UNIVERSITY OF ALBERTA

THE INFLUENCE OF NUTRITION, GROWTH AND BODY COMPOSITION ON ENDOCRINE STATUS AND REPRODUCTIVE DEVELOPMENT OF GILTS

BY

©

EDUARDO BELTRANENA MEJICANO

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

IN

ANIMAL NUTRITION / REPRODUCTION

DEPARTMENT OF ANIMAL SCIENCE
EDMONTON, ALBERTA
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ISBN 0-315-73088-9

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>
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> Beltranena, E., A. L. Schaefer, F. X. Aherne, and G. R. Foxcroft. Metabolic and reproductive status of pre-pubertal gilts treated with exogenous porcine somatotropin. Submitted to the J. Anim. Sci. in March 1992.

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DEGREE: Doctor of Philosophy

YEAR THIS DEGREE GRANTED: 1992

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ABSTRACT

The effects of feeding level on body weight (BW), growth rate (GR), fatness (BF/BW) and ovulation rate (OR) at first (puberty) and second estrus were examined in 145 gilts. From 47.2 kg to second estrus, gilts were fed 2.0 kg/d (LL) or were allowed ad libitum access to feed (HH). From first (puberty) to second estrus, half the LL gilts were fed 2.8 kg/d (LH). A quadratic negative relationship between GR and age at puberty indicated that age at puberty was minimum at a lifetime GR of .60 kg/d. It was concluded that maximizing GR in replacement gilts does not hasten the attainment of puberty. Flush-feeding of LL (LH) gilts only normalized OR to that in HH gilts. Gilts in LH and HH groups had a greater proportion of samples >1 ng/mL PLH suggesting that episodic pLH secretion was increased compared with LL gilts. Increased plasma insulin in HH gilts suggested that insulin may be an important metabolic cue, which in association with gonadotropins and IGF-I, mediates the ovulatory response to flushing.

To separate the effects of fatness from those of lean tissue growth on sexual development, 52 gilts (33.2 kg) were allowed ad libitum access to feed (HE; n = 21) or were fed restrictively (LE; n = 31) until 160 d of age. Sixteen LE gilts were then realimented until 175 d (LER). Carcass fat depth was lower in LE gilts than in HE or LER gilts, but carcass lean depth was similar. Treatment did not affect pLH or GH secretion, plasma insulin or IGF-I concentrations, reproductive tract weight, follicular volume, plasma or follicular fluid estradiol-17β. Differences in fatness, in the presence of similar lean accretion rates therefore did not influence sexual development in the pre-pubertal gilt.

To study the effects of pST administration on reproductive development, 23 gilts (61.7 kg) served as untreated controls (n = 8), or were injected (2 mg/d; n = 8) or implanted (2 mg/d; n = 7) with pST, for 28 d and allowed ad libitum access to feed. Although pST administration had no effect on GR, feed conversion or carcass characteristics, basal and episodic GH secretion were suppressed while plasma insulin and plasma and follicular IGF-I were elevated. Secretion of pLH, reproductive tract weight, follicular volume, plasma and follicular fluid estradiol-17β were similar among groups. Therefore, administration of pST did not impair reproductive development in pre-pubertal gilts.

ACKNOWLEDGEMENTS

I am greatly indebted to Dr. George R. Foxcroft and Dr. Frank X. Aherne for their guidance, patience and support throughout my graduate program and for showing me what excellence in research means. Also, I would like to thank Dr. Roy N. Kirkwood and Dr. Al L. Schaefer for their constructive criticism of my work and their camaraderie. Thanks are due also to Dr. Ray H. King, Dr. Robert J. Christopherson and Dr. Vicki Harber, members of my examination committee, and to Dr. John P. Bowland, who reviewed the final manuscript of this thesis; their comments were fair and constructive.

Without the tenacious assistance of Ms. Cheryl Streeter, Ms. Shirley Shostak, Ms. Madhu Garg, Ms. Dorothy Payne, and Ms. Lynn Wright, I would not have gotten through all those RIA hormone assay tubes. A special acknowledgement is extended to Mr. Gordon Grant and Ms. Nancy and Dr. Frank Robinson for their guidance during my initiation to radioactive isotopes and RIA.

The terrific aid of Mr. Bob Mascarin, Mr. Keith Moss and Mr. Ed Maycher in feeding and caring of all animals involved in my many experiments at the University's Piggery, is greatly appreciated. Similarly, I acknowledge Ms. Laverne Holt, Ms. Lynda Nicholls and the personnel of the Piggery and Meat Lab at the Agriculture Canada Lacombe Research Station for their assistance with the pST experiment (Chapter V). Thanks are due also to Ms. Dorothy Irwin who cared for the cannulated gilts, and whose cheerful temperament kept us going, making the long hours of bleedings much shorter. For their skilful assistance during surgery, I thank Ms. Brenda Tchir and Ms. Charlene Gorsak, and to Mr. Jack Francis, who built our surgery tables and recovery pens.

For showing me not only the how, but also the why around the lab, I am grateful to Mr. Terry and Ms. Mijarna Fenton, Mr. Brian Turner and the lab personnel, and to Ms. Anya Nowinska for ordering all research supplies and her constant bugging. To Mr. Ray Weingardt who with unlimited patience taught me the secrets of biometrics, and to Mr. Brian Kerrigan and Mr. Gavin Godby for nourishing my love for computers and software, I express my thanks.

Because we went through together and because they supported and advised me, I thank my research group colleagues Dr. Andrew Pharazyn, Ms. Sue Charlton, Ms. Emma Clowes, Dr. John Cosgrove, Ms. Teresa Wiesak and Mr. Jianchi Ding. I also thank my friends Dr. Erasmus Okine, Mr. Sam Harbison, Dr. Sam Baidoo, Dr. Tomas A. Scott and Mr. Gordon Grant who were there to listen and advise me throughout these years. And to Dr. Alfredo Newell and Dr. Enrique Hernandez whose erudition steamed my initial interest in science and research.

With great perseverance and oblation, my parents have continuously supported and encouraged my pursuit for higher education; to them, I will be eternally grateful. Also to my Agueli and clan Beltranena, I felt them always close although they were far away. And last, but always with me, to Cathy whose advocation got me to conclude this degree.

Finally, the financial support for these studies by the Natural Science and Engineering Council of Canada, the Alberta Pork Producers Development, the Alberta Agriculture Research Institute, the Department of Animal Science and Faculty of Graduate Studies and Research, is greatly appreciated.

TABLE OF CONTENTS

Page

I. INTRODUCTION 1

A. Problem Demarcation 1

Sexual Precocity (1); Reduced Litter Size (2); Longevity and Productivity (2)

B. Sexual Development Leading to Puberty in the Replacement Gilt 3

Ovarian Follicular Development (3); Developmental Pattern of Gonadotropin Secretion (4); The Estrogen/Progesterone Feedback Mechanism (5); Theories on the Onset of Puberty (6)

C. Effects of Body Weight, Body Composition and Growth Rate on Sexual Development and the Attainment of Puberty 8

Body Weight and Growth Rate (8); Body Composition (9); Tissue-Repartitioning Agents and Sexual Development (11); Nutrition, Metabolic State and Sexual Development (11); Nutrition and Ovulation Rate (12)

D. Objectives 13

E. Literature Cited 15

II. EFFECTS OF PRE- AND POST-PUBERTAL FEEDING ON PRODUCTION TRAITS AT FIRST AND SECOND ESTRUS IN GILTS 22

A. Introduction 22

B. Experimental Procedure 23

Page

Animals and Treatments (23); Measurements (24); Statistical Analyses (25)

C. Results 26

Animals (26); Feed Intake (26); Production Traits (27); CA*CL Test (27); Correlations Between Production Traits at Puberty (28); Correlations Between the Increase in Ovulation Rate from First to Second Estrus and Age, Body Weight, Backfat Thickness, Growth Rate and Fatness at Second Estrus or Their Change Between First and Second Estrus (29)

D. Discussion 29

Feed Restriction and the Attainment of Puberty (29); Body Weight at Puberty (29); Growth Rate and the Attainment of Puberty (30); Body Fatness and the Attainment of Puberty (31); Nutritional Flushing (32)

E. Implications 34

F. Literature Cited 39

III. ENDOCRINOLOGY OF NUTRITIONAL FLUSHING IN GILTS 42

A. Introduction 42

B. Experimental Procedure 43

Animals and Treatments (43); Cannulation and Bleeding (44); Radioimmunoassays (45); Statistical Analyses (47)

C. Results 49

Page

Feed Intake (49); Ovulation Rates (49); Changes in Plasma pLH and FSH (50); Changes in Plasma Estradiol-17β and Progesterone (50); Changes in Plasma Insulin and IGF-I (51)

D. Discussion 51

Gonadotropin Involvement in Flushing (51); Insulin and IGF-I Involvement in Flushing (53)

E. Implications 55

F. Literature Cited 61

IV. INNATE VARIABILITY IN SEXUAL DEVELOPMENT IRRESPECTIVE OF BODY FATNESS IN GILTS 66

A. Introduction 66

B. Experimental Procedure 67

Animals and Treatments (67); Diets (68); Cannulation, Bleeding and Slaughter (68); Measurements (69); Radioimmunoassays (70); Statistical Analyses (75)

C. Results 76

Animals (76); Live Weight, Backfat Thickness and Energy Intake (76); Carcass Measurements (77); Reproductive Status (77); Metabolic Status (78)

D. Discussion 79

Effectiveness of the Dietary Regimens (79); Effect on Hormonal Metabolic Status (79); Effect of Fatness on Reproductive Status (81); Innate Variability

Page

in Sexual Development Irrespective of Body Fatness and(or) Dietary Regimen (83)

E. Implications 84

F. Literature Cited 96

V. METABOLIC AND REPRODUCTIVE STATUS OF PRE-PUBERTAL GILTS TREATED WITH EXOGENOUS PORCINE SOMATOTROPIN 100

A. Introduction 100

B. Experimental Procedure 102

Animals, Treatments and Diet (102); Housing, Surgery and Bleeding (102); Measurements (103); Radioimmunoassays (104); Statistical Analyses (106)

C. Results 107

Growth Traits and Carcass Characteristics (107); Metabolic Status (107); Reproductive Status (109)

D. Discussion 110

Efficacy of Drug Administration (110); Effect of pST on Growth Hormone Episodic Secretion (111); Hyperinsulinemia Caused by pST Administration (111); Effect of pST on Reproductive Development (113)

E. Implications 116

F. Literature Cited 124

Page

VI. GENERAL DISCUSSION AND CONCLUSIONS 130

A. Literature Cited 138

LIST OF TABLES

Table Page

II.1 The effect of pre- and post-pubertal level of feeding on production traits 35

II.2. Correlation coefficients (r) between various production traits at puberty 36

III.1. The effect of pre- and post-pubertal level of feeding on ovulation rate at first and second estrus 56

IV.1. Low-energy (LE) and high-energy (HE) diets 85

IV.2. Effects of dietary regimen on growth traits and carcass measurements of pre-pubertal gilts 86

IV.3. Effects of dietary regimen on characteristics of episodic LH secretion in pre-pubertal gilts (all gilts) as established using sliding window analysis 87

IV.4. Effects of dietary regimen on characteristics of LH secretion in pre-pubertal gilts (low-frequency only) as established using cluster analysis 88

IV.5. Effect of dietary regimen on characteristics of reproductive development in pre-pubertal gilts 89

IV.6. Effects of dietary regimen on characteristics of episodic GH secretion in pre-pubertal gilts as established using sliding window analysis 90

IV.7. Effect of dietary regimen on characteristics of GH secretion in pre-pubertal gilts as established by cluster analysis 91

V.1. Effects of pST administration for 27 days on growth traits and carcass characteristics in pre-pubertal Yorkshire gilts 117

Table Page

V.2. Effects of pST administration for 23 days on characteristics of episodic GH secretion (cluster analysis) of pre-pubertal Yorkshire gilts 118

V.3. Effects of pST administration for 23 days on characteristics of episodic GH secretion (sliding window analysis) of pre-pubertal Yorkshire gilts . . 119

V.4. Effects of pST administration for 23 days on characteristics of episodic LH secretion (cluster analysis) of pre-pubertal Yorkshire gilts 120

V.5. Effects of pST administration for 23 days on characteristics of episodic LH secretion (sliding window analysis) of pre-pubertal Yorkshire gilts 121

V.6. Characteristics of reproductive development in pre-pubertal Yorkshire gilts after pST administration for 27 days 122

LIST OF FIGURES

Figure Page

II.1. Pooled daily feed intake for HH gilts in experiments 1, 2, and 3 37

II.2. Relationship between age at puberty and several production traits at puberty 38

III.1. Least-square means for the main effect of time irrespective of feeding level on gonadotropin plasma concentrations through the second peri-estrous period. 57

III.2. Least-square means and standard errors for feeding level x time (day) for pLH during the pre-surge period. 58

III.3. Least-square means and standard error for feeding level x time of the day on pre- and post-prandial plasma insulin through the second peri-estrous period. 59

III.4 Least-square means for plasma IGF-I during the pLH surge period. . . . 60

IV.1. Weekly least-square means for body weight gain and backfat thickness gain and daily energy intake of pre-pubertal gilts. 92

IV.2. Two littermate gilts, one with a high-frequency and one with a low-frequency of episodic LH secretion 93

IV.3. Relationships between reproductive tract weight (RTW), total follicular volume (TFV) of the 10 largest follicles and indicators of basal and episodic LH secretion 94

Figure Page

IV.4. Least-square means for plasma insulin (INS), insulin-like growth factor I (IGF-I) and non-esterified free fatty acids (NEFA) concentrations for LE and HE gilts at 158 days of age, and for LER gilts at 173 days of age. . 95

V.1. Hourly mean plasma concentrations of growth hormone (GH), insulin (INS), insulin-like growth factor I (IGF-I) and non-esterified free fatty acids (NEFA) during the pre-prandial, post-prandial and post-injection periods on day 23 of treatment in untreated control and pST-treated gilts. 123

I. INTRODUCTION

A. Problem Demarcation

Management of the replacement gilt and her successful integration into the breeding herd continue to present severe problems to the swine industry. Presently, sow culling rates are high, with most sows being culled before they achieve their potential optimum performance. Sow culling rates ranging from 45 to 70% (Dial, 1992), indicate that an equivalent number of replacement gilts must be brought into the herd annually. However, replacement gilts have lower productivity, and at best will merely maintain herd performance. Thus, when the goal is to optimize the herd's reproductive performance, productivity of the replacement gilt is crucial to the herd's profitability. Nonetheless, several factors affect productivity of the replacement gilt, and therefore, the efficiency with which they are brought into the herd:

Sexual Precocity. Ideally, the replacement gilt must be pregnant at that age equivalent to the herd's average age at market weight. The number of days between mean age at market weight and age at which the replacement gilt first becomes pregnant, has housing, management and feeding costs associated with it that ought to be minimized. This period of time is comparable to the weaning to mating interval, but even in the majority of well managed, established herds, it exceeds the weaning to mating interval, not merely by several days, but by several weeks. Minimizing age at the attainment of puberty provides the opportunity for an equivalent reduction in age at first mating. Achievement of this objective, however, is limited by factors that influence the attainment of puberty in the replacement gilt. Some of these factors or cues are intrinsic or genetically-determined, henceforth referred as "internal" and include body weight, body fatness, and growth rate. Others, in contrast, are environmental cues, henceforth referred as "external", which influence the expression of the genetically-determined internal factors and include nutrition, boar stimulation, confinement, photoperiod, etc. The interaction between external factors, mainly nutrition, and the genetically-determined internal factors are the main topic of this dissertation; their importance will be further emphasized later in this chapter.

Reduced Litter Size. Primiparous sows tend to have smaller litters than older, multiparous sows (Aherne and Kirkwood, 1985). A reduced litter size suggests that replacement gilts may have lower ovulation rates, larger embryo mortality or a combination of these two (Aherne and Kirkwood, 1985; Dyck, 1988). Nutritional management to minimize embryo mortality in the first parity gilt in particular, requires careful consideration (see Pharazyn, 1992). The subject of ovulation rate and nutritional flushing are also part of this dissertation and will be further emphasized later in this chapter.

Longevity and Productivity. Modern sow productivity is based on longevity rather than annual productivity. Nonetheless, a large component of a herd's annual culling rate, comprises culling of first and second parity sows. High removal rates after the first or second parity are frequently associated with low birth rates, reduced milk production, excessive weight loss during lactation and extended weaning to mating interval. The physiological correlates of these phenomena will not be discussed here, but require careful consideration and the development of management practices to avoid such apparent metabolic exhaustion early in the productive life of the sow (see Lythgoe, 1987; Williams and Mullan, 1989; Baidoo, 1989).

B. Sexual Development Leading to Puberty in the Replacement Gilt

Puberty is associated with the development and activation of the hypothalamic-hypophysial-gonadal axis. In order to place in context the genetically-determined traits and environmental factors that influence sexual development and the attainment of puberty, it is necessary, first, to briefly describe the endocrine correlates leading to the natural attainment of puberty. Several reviews have recently examined this subject in the replacement gilt (Elsaesser, 1982; Ellendorff and Parvizi, 1982; Baird, 1985; Dyck, 1988; Short et al., 1988; Foxcroft et al., 1989); based on these, a brief summary is as follows:

Ovarian Follicular Development. Around day 30 of gestation, the porcine fetal gonads differentiate to an ovary, which contains egg nests. The number of egg nests however, declines with the advance of fetal development, and they are seldom observed in the 20 day-old female. From approximately day 70 of gestation to day 70 after birth, primordial follicles are the dominant oogonic structures in the ovary. Primary follicles are first observed by day 70 of gestation. Secondary follicles are usually observed by the time of birth, and constitute about 30% of the total oogonic structures by day 90 after birth. Tertiary or antral follicles, which are gonadotropin-sensitive, are not commonly present in the 70 day-old growing gilt (see Pressing et al., 1992). Measurable concentrations of estradiol and progesterone observed in the gilt at birth are not of follicular origin, but from maternal-placental sources, and are usually undetectable five days after birth.

Developmental Pattern of Gonadotropin Secretion. Prior to day 80 of fetal life, gonadotropin secretion is undetectable; thereafter both circulating FSH and LH concentrations increase and are highest about the time of birth. During infancy and the early juvenile periods, they follow divergent patterns; FSH remains elevated while LH declines to very low levels. It appears that exposure to comparatively high levels of FSH results in the development of gonadotropin receptors in antral, tertiary follicles. Once the gonadotropin receptors have been established, maturation of the first wave of follicles appears to be dependent upon the frequency of episodic LH secretion. The decline in circulating FSH concentrations observed in the mid pre-pubertal period, appears to be related to a follicular selective suppressive mechanism, possibly inhibin produced by the growing follicles; however, this decline in FSH secretion has also been reported in the ovariectomized gilt, which suggests the involvement of a central rather than a follicular suppressive mechanism. Opioid peptides have been suggested as possible central selective regulators of gonadotropin secretion (see Trudeau et al., 1988; Trudeau et al., 1989).

Episodic LH secretion in the gilt, as in other species, closely corresponds to the hypothalamic release of GnRH, which is regulated by the "pulse generator centre" located in the medio-basal hypothalamus (Knobil, 1989). During the early pre-pubertal period, there appear to be well-defined, large LH episodes occurring at a rate of 1/1.5 hours. However, during the late pre-pubertal period, there appears to be an increase in episodic frequency and a decline in episodic amplitude, which appear to be more evident during the night time (see Cosgrove, 1991). Another characteristic of the late pre-pubertal period in the gilt is a diurnal rhythm in episodic LH secretion; night-time frequency is increased with respect to day-time frequency (see Booth 1990b; Cosgrove, 1992).

The Estrogen/Progesterone Feedback Mechanism. As the growing follicles mature, circulating levels of estrogens increase, suppressing LH pulse amplitude. This elevation in circulating estrogens, mainly estradiol-17β, ultimately acts on the central nervous system (CNS) to cause behaviourial pubertal estrus and on the combined hypothalamic-hypophysial axis to cause the pre-ovulatory LH "surge" and first ovulation. This stimulatory effect of ovarian steroids on the hypothalamic-hypophysial axis has been termed "positive feedback". The mature follicles in turn respond to this LH surge by ovulating, while the remaining granulosa and theca tissue of the follicle undergoes luteinization to form the corpora lutea and the synthesis and release of progesterone increases. Progesterone modulates gonadotropin release by the hypothalamic-hypophysial axis, preventing the development of highly estrogenic follicles, an essentially "negative feedback" effect. Finally, if fertilization and implantation do not take place, luteal function is terminated by uterine prostaglandins, which induce luteolysis of the corpora lutea thereby removing the inhibitory effect of progesterone on the hypothalamic-hypophysial axis.

Theories on the Onset of Puberty. Two hypotheses have been commonly proposed as explanations for the mechanism involved in the control of the onset of puberty (see Paterson, 1982). Firstly, since a smaller dose of estrogen suppresses circulating levels of gonadotrophin in immature rats as compared with adults, a theory of "differential sensitivity to ovarian steroids" or "gonadostat theory" was proposed. According to this theory, puberty occurs when the hypothalamic pulse generator centre that controls gonadotrophin release becomes desensitized to steroid inhibition during sexual maturation. The second theory postulates, that "establishment of the positive feedback system" is responsible for the onset of puberty. This theory is based upon evidence that estrogen treatment does not induce an LH surge in pre-pubertal monkeys and humans. Neither of these theories explains the increase of gonadotrophins observed in neonatally-gonadectomized female animals at the time at which puberty would normally occur and during adolescence in agonadal humans with Turner's {X_} and Klinefelter's syndrome {XXY} (see Adams and Steiner, 1988).

Accumulating evidence in the literature suggests that the key events controlling the onset of puberty are not solely dependent on changes in gonadal steroid feedback but on the maturation of the more complex hypothalamic mechanisms controlling the release of GnRH (see Karsch, 1987). Pulsatile administration of GnRH induces precocious puberty in immature gilts (see Pressing et al., 1992). When the pituitaries of immature rats are transplanted to mature hypophysectomized-rats, or the gonads of immature rats are transplanted to mature gonadectomized-rats normal reproductive activity has been reported. The anterior pituitary gland or adenohypophysis and the ovary or testis, therefore, are capable of becoming functional before puberty. Consequently it has been proposed that, it is indeed the hypothalamus which regulates the onset of puberty (Clarke, 1988). Besides, lesions of the posterior hypothalamus advance the onset of menarche and first ovulation, while neither pregnant-mare serum gonadotrophin (PMS) and/or human-chorionic gonadotrophin (hCG) treatment, nor repeated estrogen injections or combinations of these exogenous hormonal treatments, resulted in precocious puberty in rhesus macaque monkeys (Terasawa et al., 1983).

That gonadal steroid feedback affects the hypothalamic centre controlling GnRH release must not be ruled out however (see Karsch, 1987). The lack of negative feedback of ovarian steroids during the neonatal and infancy periods may be due to a lack of ovarian secretory activity per se. Species and sex differences do, in fact, exist. In lambs, the feedback mechanism seems to develop early in life as compared with other species (Foster et al., 1988). Young male pigs differ from their female contemporaries in that the testes appear to be active at an earlier age than the ovaries. Maturation of the positive feedback mechanism early in life may not be essential; nonetheless, during the peri- and post-pubertal periods such a mechanism may indeed affect the hypothalamic-hypophysial release of gonadotrophin.

To conclude, while the initial stage of sexual development may be solely dependent on the onset of pulsatile release of GnRH, the establishment of a gonadal steroid positive feedback mechanism at puberty is imperative for the acquisition of full reproductive capacity.

C. Effects of Body Weight, Body Composition and Growth Rate on Sexual Development and the Attainment of Puberty

As mentioned earlier, several environmental cues or "external" factors interact with genetically-determined "internal" factors to influence sexual development and the attainment of puberty. The objective of this section is not to present an exhaustive review, but rather to summarize relevant information on how body weight, body composition and growth rate may influence or signal maturation of the reproductive axis. An attempt will be made to dissociate and examine individual factors, although the complex and additive interactions among them, must be kept in mind.

Body Weight and Growth Rate. Various studies have shown either a direct, an inverse or no relationship between body weight or growth rate and the attainment of puberty (see Anderson and Melampy, 1972; den Hartog and van Kempen, 1980; Hughes, 1982; Knott et al., 1984). Because most of these studies (see Anderson and

Melampy, 1972; den Hartog and van Kempen, 1980; Hughes, 1982; Knott et al., 1984) used different planes of feeding and different age at boar stimulation, the relationship between body weight or growth rate and attainment of puberty are totally confounded. Thus it can be expected that gilts which have attained a certain degree of sexual maturity, irrespective of body weight or growth rate, may respond equally fast to boar stimulation. It has been suggested, however, that there is a threshold or minimum weight, probably associated with a certain degree of somatic growth, that gilts need to achieve in order to attain puberty (Kirkwood and Aherne, 1985; Kirkwood et al., 1987). However, once gilts surpassed this yet undetermined threshold value, body weight becomes relatively unimportant to the attainment of puberty, and other factors appear to withhold the attainment of puberty (i.e., boar stimulation, stress). Thus, neither body weight nor age at puberty, are reliable indicators of sexual development.

Body Composition. Frisch (see Frisch, 1988) suggested that there was a relationship between body weight and the age at which girls achieved menarche. Later, it was suggested that body fatness explained the onset of menarche better than did body weight, and this theory was subsequently refined to a ratio of fat:lean body mass, which not only appeared to explain onset of menarche, but also amenorrhea observed in women suffering anorexia nervosa and women who exercised strenuously (Frisch, 1988). Based on the Frisch model, Kirkwood and Aherne (1985) extrapolated the theory to swine. They suggested that a minimum level of fatness may be required for the attainment of puberty in the replacement gilt. They also suggested that the level of fatness could be a better indicator of reproductive development than age, body weight or growth rate. Kirkwood et al. (1987) suggested later that, as for body weight, a minimum level of fatness or fatness threshold may be required or permissive for puberty to occur, but was not, in itself, sufficient to trigger the onset of puberty. Several reports are consistent with this theory in swine, since it appears that fast-growing, lean gilts may attain puberty at an older age than their slow-growing, fatter counterparts (Reutzel and Sumption, 1968; Price et al., 1981). Although not proven yet, concern has been expressed that fast-growing gilts may also have a decreased propensity to fatten, therefore, sexual development and the attainment of puberty could be delayed, even though the age and weight thresholds have been exceeded (Kirkwood and Aherne, 1985). Further concern has been expressed that there has been a considerable reduction in fatness in market weight hogs (see Aherne and Kirkwood, 1985), and this could be indicative of future problems linking body fatness and reproductive activity in gilts and sows.

Body fatness may also influence reproduction through steroid metabolism. It appears that body fatness may hold a significant pool of progesterone, which is capable of aromatizing androgens to estrogens, and altering the ratio of free steroid:sex hormone-binding globulins (Kirkwood and Aherne, 1985; Kirkwood et al., 1987; Frisch, 1988; I'Anson et al., 1991). Most of these studies have been conducted in adult and(or) obese humans, and therefore the possibility exists that, because of reduced body fatness in the pre-pubertal animal, fatness may have little influence on steroid metabolism. Other reports suggest that in the gilt, body weight and fatness are also associated with an alteration in the excretion of estrogens, and as the gilt matures, there is a decrease in the metabolic clearance rate of estradiol (Elsaesser et al., 1982).

Tissue-Repartitioning Agents and Sexual Development. Driven by a demand for leaner pork, modern swine production will probably involve the use of tissue-repartitioning agents such as beta-agonists and recombinant porcine somatotropin (pST). Administration of such drugs can result in drastic reduction of body fatness with a concomitant increase in lean tissue accretion (Chung et al., 1985; Campbell et al., 1989). Concern has been expressed, that if replacement gilts have previously been treated with these drugs, achievement of the permissive fat:lean ratio required for sexual development and attainment of puberty will be even more difficult to achieve. Furthermore, it has not been established what effects these drugs may have irrespective of changes in body composition. Reports have already been published suggesting that pST-treated gilts may not achieve puberty while receiving the drug, but that following withdrawal, normal attainment of puberty is possible (Bryan et al., 1989; Bryan et al., 1990). Other reports have suggested that pST treatment may impair follicular steroidogenesis (Hagen et al., unpublished observations; Hagen et al., 1990). Thus, there is an urgent need to clarify the effects of tissue-repartitioning agents on sexual development.

Nutrition, Metabolic State and Sexual Development. It has long been recognized in several species that because the reproductive axis has a low priority for nutrients, sexual development and attainment of puberty can be delayed by poor nutrition (Steiner et al., 1983; Foster et al., 1988; I'Anson et al., 1991). Nutritional insult results in alterations in metabolic state: Armstrong and Britt (1987) have shown that pulsatile secretion of GnRH in the gilt may be switched on and off by feed restriction and realimentation; Booth (1990b) has also shown that acute feed restriction followed by realimentation in the absence of changes in body composition results in alteration in the frequency of episodic LH secretion, and finally, Cox et al. (1987) have shown that adminstration of exogenous insulin increased ovulation rate. Although not confirmed in swine, Steiner et al. (1983) and Downing and Scaramuzzi (1991) have shown that dietary intake of certain amino acids may alter episodic LH secretion in the Macaque monkey and insulin concentrations in sheep, respectively. Thus, plane of feeding and diet composition can alter certain metabolic signals that can influence the maturation of the hypothalamic-hypophyseal-ovarian axis. Roles for plasma amino acids, free fatty acids, glucose, growth hormone, thyroxine and insulin-like growth factor I (IGF-I) have been proposed as mediators or indirect regulators of the nutritional effects on the reproductive axis in swine (Booth, 1990a). Thus, it appears that the hypothalamus of the growing animal is capable of monitoring some metabolic parameters, which are used to time the onset of puberty (I'Anson et al., 1991).

Nutrition and Ovulation Rate. Restricting feed or energy intake before puberty, or between estrous cycles, reduces ovulation rate in gilts; however, short-term, high-level feeding during the first estrous cycle (flushing), increases ovulation rate (Anderson and

Melampy, 1972; den Hartog and van Kempen, 1980). It is not clear, however, whether flushing merely reverses the negative effect of nutritional restriction on ovulation rate or induces superovulation (Aherne and Kirkwood, 1985). The physiological mechanisms, by which an increase in feeding restores ovulation rate, have yet to be established (see Rhodes et al., 1987; Hughes and Pearce, 1989). Since it has been shown that chronic, severe feed restriction impairs the hypothalamic GnRH pulse generator (Armstrong and Britt 1987) and that a rapid increase in pulsatile LH secretion occurs after realimentation (Flowers et al., 1988; Booth 1990b; Cosgrove, 1992), it appears that gonadotropins mediate the restoration of ovulation rate. Cox et al. (1987) have established that administration of exogenous insulin increases ovulation rate, and provides preliminary evidence that insulin affects both gonadotropin secretion and the ovary directly by altering follicular atresia.

D. Objectives

The objective of the studies described herein were:

In the first study, to further examine the effects of pre-pubertal feeding level on body weight, fatness, growth rate, and age at the attainment of puberty. Also, to evaluate the effect of pre- and post-pubertal feeding level on ovulation rate at first and second estrus in gilts.

In the second study, to compare the endocrine status of gilts fed restrictively, flushed and allowed ad libitum access to feed throughout the second peri-estrus period, and thereby provide additional in vivo confirmation of potential mediators of the ovulatory response observed in flushing.

In the third study, to examine the effects of manipulation of body fatness on the activity of the hypothalamic-hypophyseal-ovarian axis in relation to the attainment of puberty in littermate gilts achieving maximal protein deposition rates, but in which fat deposition rate was altered.

And in the fourth study, to examine the physiological mechanism(s) by which porcine somatotropin (pST) might affect sexual development and the attainment of puberty in the replacement gilt.

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II. EFFECTS OF PRE- AND POST-PUBERTAL FEEDING ON PRODUCTION TRAITS AT FIRST AND SECOND ESTRUS IN GILTS[1]

A. Introduction

Demand for leaner pork has forced swine breeders to continuously select pigs for reduced body fatness while continuing to select for improvements in growth rate. Consequently, the ratio of lean tissue to fat is being altered. If a permissive lean:fat ratio is required for the onset of puberty (Kirkwood and Aherne, 1985; Kirkwood et al., 1987), such a selection trend is likely to retard the attainment of puberty because this ratio will take longer to be achieved. Reproductive efficiency is also affected in that leaner gilts farrow at an older age (Johansson and Kennedy, 1983). Furthermore, this selection trend may lead to females with a large mature body size, with a consequent increase in energy maintenance requirements. Thus, the associations among growth rate, body weight and fatness and the attainment of puberty in genetically lean gilts need to be re-evaluated.

Restricting feed or energy intake before puberty, or between estrous cycles, reduces ovulation rate in gilts. Nonetheless, short-term, high-level feeding during the first estrous cycle (flushing), increases ovulation rate (Anderson and Melampy, 1972; den Hartog and van Kempen, 1980). It is not clear, however, whether flushing merely reverses the negative effect of nutritional restriction on ovulation rate or induces superovulation (Aherne and Kirkwood, 1985).

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[1] A version of this chapter has been published. E. Beltranena, F. X. Aherne, G. R. Foxcroft and R. N. Kirkwood. J. Anim. Sci. 1991. 69:886.

The objectives of this study were, first, to examine the effects of pre-pubertal feeding level on body weight, fatness, growth rate, and age at the attainment of puberty; and second, to evaluate the effect of pre- and post-pubertal feeding level on ovulation rate at first and second estrus in gilts.

B. Experimental Procedure

Animals and Treatments. One hundred and forty-five (Yorkshire x Landrace) crossbred pre-pubertal gilts were used in three replicate experiments conducted during the summer and fall of consecutive years. The gilts were weaned at 28 days of age, thereafter penned in groups and allowed ad libitum access to commercial starter and grower diets. After allocation to the experimental treatments, gilts were fed a barley, wheat, and soybean meal-diet formulated to provide a minimum of 16% crude protein, .95% lysine, 13.1 MJ DE/kg and to exceed the NRC (1979) recommended requirements for growing pigs for amino acids, vitamins and minerals. Gilts fed restrictively were penned in groups of four but were individually fed three meals of approximately equal size at 0630, 1130 and 1530 h. Gilts with ad libitum access to feed also were penned in groups of four but had continuous access to the diet from self-feeders.

From 47.2 SD .6 kg body weight and 98.2 SD .5 days of age, until three days after the attainment of first estrus (puberty), sets of gilts with similar post-weaning growth rates (calculated as current body weight minus birth weight divided by age using only animals within ± 1.8 x SD), were assigned randomly to be fed either 2.0 kg/day (L, n = 86) or were given ad libitum access to feed (H, n = 59). From three days after the attainment of first estrus until five days after the attainment of second estrus, the feed allowance of one-half of the gilts in the L group was increased to 2.8 kg/day (LH, n = 43); the other L gilts continued to be fed 2.0 kg/day (LL, n = 41). All gilts in the pre-pubertal H group continued feeding ad libitum (HH, n = 46) until five days after the attainment of second estrus.

From an average pen body weight of 65 kg until five days after the individual attainment of second estrus, gilts were moved daily as a group into one of three or four mature boar pens on a rotational basis for stimulation and detection of estrus. Gilts were in full body contact with boars for approximately 20 minutes and subsequently were returned to their home pens. Any gilt showing estrus was removed immediately from the boar's presence in order to prevent mating.

Measurements. Body weight was recorded weekly for all gilts in the three experiments. Backfat thickness was measured (Scanoprobe II, Ithaca, NY) at the same time as body weight in all gilts in experiments 2 and 3. Probe sites were aligned with the apex of the curve of the last rib, at both sides, 5 centimetres off the midline and were permanently marked with a tattoo for subsequent readings. Gilts that showed normal estrous activity were slaughtered five to seven days after the onset of their second estrus. Any gilt that had not shown behaviourial second estrus 30 days after the date of onset of first estrus was slaughtered. After slaughter, reproductive tracts were collected and ovaries were removed at the hilus. The ovaries were then sliced in approximately 1-mm sections. Ovulation rate at first and second estrus was established by counting corpora albicantia (CA) and corpora lutea (CL), respectively. In experiment 1, in order to substantiate this method for determining ovulation rates in consecutive estrous cycles in the same animal, the number of corpora albicantia counted at slaughter after the second estrus in HH gilts was compared with counts of corpora lutea following the slaughter of 11 H gilts five days after their pubertal estrus. Hereafter, this will be referred as the "CA*CL test".

Statistical Analyses. Statistical analyses were performed using the General Linear Models procedures of SAS (1988). Differences in age, body weight, backfat thickness and ovulation rate were assessed by unbalanced analysis of variance of a split-plot design. Overall treatment effects were established using the error term of gilts nested within year x treatment. Multiple comparison for interactions (feeding level x time [first to second estrus]) were performed according to the method of Harter (1970). Tukey's studentized range test was used to compare means. Associations between variables were explored using Pearson correlation and regression analyses with correlation coefficients first calculated separately for each year and treatment and then pooled. Student's t-test was conducted to examine the hypothesis of difference in the estimation of pubertal ovulation rate by counting corpora lutea versus corpora albicantia for the CA*CL test.

C. Results

Animals. Four of the 145 gilts were withdrawn from the experiments before the attainment of puberty due to unsatisfactory growth rate (2 L) and leg weakness (2 H). In addition, the data from 11 H gilts involved in the CA*CL test, data from three gilts injured by the boars (2 LH, 1 HH) and 12 gilts slaughtered 30 days after puberty having not shown second estrus, were excluded from statistical analysis of traits at second estrus. Post-mortem examination of the reproductive tract morphology of these anestrous gilts revealed a gravid uterus in four (2 LL, 2 HH) and cystic follicles in the ovaries of two (1 LL, 1 LH); the presence of corpora lutea and corpora albicantia in the ovaries of one (1 LH) gilt suggested that a second estrus had gone undetected. The absence of corpora lutea in the ovaries of the remaining five gilts indicated true post-pubertal anestrus (4 LL, 1 LH). Consequently, data from 141 gilts (84 L, 57 H) were used in regression and correlation analysis of traits at puberty. However, complete data sets from only 115 gilts were available for a statistical analysis of changes in a variable from first to second estrus (34 LL, 38 LH and 43 HH).

Feed Intake. The pooled pattern of voluntary feed intake for the HH gilts is show in Figure II.1. Feed intake for the H gilts at initiation of the study was similar to the feeding allowance of the L gilts. At first estrus, feed intake for the H gilts was 3.4 kg/day, equivalent to 170% of the feeding allowance of the L gilts. And at second estrus, feed intake for the HH gilts reached 3.6 kg/day, equivalent to 190 and 135% of the feeding allowance for the LL and LH gilts, respectively.

Production Traits. Table II.1 presents the least-square means of the pooled data for age, body weight, ovulation rate and backfat thickness. These data were pooled because there was no significant effect of year nor were there significant interactions for year x feeding level, year x time (first, second estrus) or year x feeding level x time ($P > .05$).

The main effect of time (first, second estrus), irrespective of feeding level, was significant for all studied traits. Gilts were older, heavier, fatter and had higher ovulation rate at second than at first estrus.

The main effect of feeding level, irrespective of time, was significant for age, backfat thickness and ovulation rate. The HH gilts were younger and fatter than either the LL or LH gilts. Gilt in the L group that had their feed allowance increased (LH) and HH gilts had greater ovulation rate than LL gilts.

Feeding level x time interactions were significant for body weight, backfat thickness and ovulation rate only. Body weight gain between first and second estrus for the HH gilts was almost twice that of the LL gilts; the LH gilts exhibited intermediate body weight gain. Gain of backfat thickness between first and second estrus was greater for the HH gilts than for either LL or LH gilts. Increase in ovulation rate between first and second estrus was greater for HH and LH gilts than for LL gilts.

*CA*CL Test.* Pubertal ovulation rate, estimated by counting corpora lutea following slaughter of 11 H gilts after first estrus, was not different from that estimated by counting corpora albicantia following slaughter of their contemporary HH gilts after the second estrus ($P > .05$).

Correlations Between Production Traits at Puberty. Overall and within each group, there was a linear and positive correlation between age and body weight at first estrus (Table II.2, Figure II.2a) and younger gilts were lighter at puberty. In contrast to body weight, there was overall a highly significant quadratic and negative relationship between age at first estrus and lifetime growth rate (Table II.2, Figure II.2b). This relationship was even stronger for the L gilts (Table II.2) and the greater the growth rate, the younger the gilt at puberty. This relationship was, however, nonsignificant ($P > .05$) for the H gilts.

The correlation between age and backfat thickness at first estrus was low and nonsignificant ($P > .05$). However, when fatness was expressed as backfat thickness/body weight at first estrus, a significant negative linear relationship with age was observed. Overall (Table II.2, Figure II.2c), and for each group, the fatter the gilt the younger it was at puberty.

Correlations between age and ovulation rate at first estrus were low and nonsignificant ($P > .05$).

Overall, body weight at first estrus was positively and highly correlated to lifetime growth rate and backfat thickness at first estrus. For the L gilts, however, there was no correlation between body weight and backfat thickness at first estrus ($P > .05$).

Correlations between lifetime growth rate and backfat thickness at first estrus and backfat thickness/body weight at first estrus were positive and significant overall and for the H gilts, yet were nonsignificant for the L gilts ($P > .05$).

Overall, ovulation rate was positively and highly correlated with fatness at first estrus (r = .27), yet this relationship was nonsignificant for the individual groups ($P > .05$).

Correlations Between the Increase in Ovulation Rate from First to Second Estrus and Age, Body Weight, Backfat Thickness, Growth Rate and Fatness at Second Estrus or Their Change Between First and Second Estrus. Overall, the increase in ovulation rate from first to second estrus was positively correlated with the increase in body weight between first and second estrus (r = .25) and with lifetime growth rate from birth to second estrus (r = .27). However, significant correlations were not established for the individual treatment groups ($P > .05$).

D. Discussion

Feed Restriction and the Attainment of Puberty. Whether moderate feed restriction (60 to 85% of ad libitum) affects age at puberty in gilts has been the subject of controversy (reviewed by Brooks and Cole, 1974; Hughes, 1982; Aherne and Kirkwood, 1985). In the present study, feed restriction delayed, but did not prevent the onset of puberty. After puberty was attained, the length of the first estrous cycle was neither affected by feeding level nor by indirect changes in growth rate, body weight or fatness over the first estrous cycle. Thus, mild feed or energy restriction delays attainment of puberty primarily by affecting growth rate.

Body Weight at Puberty. The close proximity in body weight at puberty between treatments groups is consistent with the suggestion that body weight may be an important determining factor in the attainment of reproductive activity (Frisch, 1988).

Similarly, van Lunen and Aherne (1987) observed no difference in body weight at puberty between gilts reared on different feeding regimens, even though growth rate and age at puberty were different.

The observed positive correlation between age and body weight at puberty (Figure II.2a) indicated that the heavier the gilt at boar stimulation, the heavier it was at puberty. This is likely a function of most gilts achieving a level of development permitting a response to boar contact. This suggestion is in agreement with the data of Burnett et al. (1988) and Knott et al. (1984). Therefore, a weight or age difference at commencement of boar contact would still be evident at puberty.

Growth Rate and the Attainment of Puberty. Of great relevance in this study is the negative quadratic relationship between age and lifetime growth rate to puberty. In contrast to this quadratic relationship, a linear and negative relationship has been reported previously (Reutzel and Sumption, 1968; Cunningham et al., 1974; Hutchens et al., 1981; Price et al., 1981; den Hartog and Noordewier, 1984; Mabry et al., 1985). The manner in which those results were analyzed, added to variability in experimental design, may have masked the existence of a quadratic relationship, in that the existence of a linear relationship may be limited to gilts fed restrictively. No relationship between age at puberty and lifetime growth rate was evident from these data (Table II.2), or those of Young et al., (1990), for gilts with ad libitum access to feed. Hence, the negative quadratic relationship observed in this study is interpreted as follows: There are at least two components in the relationship between lifetime growth rate and age at puberty (Figure II.2b). In the first, with a negative slope and mainly associated with feeding restrictively, the greater the growth rate, the younger the gilts would be at puberty. In the second, with a slope no different from zero and mainly associated with gilts given ad libitum access to feed, growth is not limiting and gilts reach puberty at the youngest age possible. Accordingly, this minimum age at puberty coincided with a lifetime growth rate of .60 kg/day ($x = -B/2C$). From the equation generated with these data, a third component seems feasible, in which the slope becomes positive and corresponds to the fastest growing gilts; in this situation age at puberty again becomes delayed as growth rate increases. Caution must be exercised however, because the number of gilts in that component was small due to the allocation procedure used in this study (only gilts within ± 1.8 x SD of post-weaning growth rate were considered). Hence, whether the slope remains zero, in which case faster-growing gilts will be heavier at puberty, or becomes positive, in which case gilts will not only be heavier but also older at puberty, is not clear.

Body Fatness and the Attainment of Puberty. Linear and positive relationships among lifetime growth rate, body weight and fatness, were noted as significant overall and for the H gilts but not for the L gilts (Table II.2). Thus, lifetime growth rate was associated with body weight at puberty but not necessarily with a certain backfat thickness or degree of fatness. The reason for this may be in the quality of tissue growth. For L gilts with a low growth rate, extremely lean growth occurred and no correlation was observed; in contrast, for H gilts, a greater growth rate may have been associated with greater fat deposition. This rationale does not, however, rule out the possibility that puberty was delayed until a certain amount of body fat or a certain degree of fatness had been achieved. Thus, for gilts within the first component of the relationship between age at puberty and lifetime growth rate, growth or some aspect of body state probably limited attainment of puberty. Similarly, for those gilts within the third component, with the fastest and possibly also the leanest growth, achievement of a certain degree of fatness also may be the factor limiting activation of the hypothalamic-hypophysial-ovarian axis. Both situations would be in agreement with the Frisch model of puberty attainment (Frisch, 1988). In contrast, for gilts within the second component, for which fat deposition is greater, neither fatness nor body weight limits attainment of puberty, because these are guaranteed by the rate and quality of growth. This seems in agreement with the conclusions of Young et al. (1990) that body weight and fatness did not limit attainment of puberty in gilts with ad libitum feed intake. Growth-independent effects on the maturation of the hypothalamic-hypophysial-ovarian axis seem, therefore, to be limiting puberty attainment in gilts within the second component.

Nutritional Flushing. Dyck (1971) suggested that only the number of corpora lutea will provide accurate estimates of ovulation rate. It is possible that when the ovary is examined superficially, follicular growth and new corpora lutea may mask the regressing corpora albicantia from the ovarian surface. Slicing of ovarian tissue accordingly is a more precise method of establishing ovulation rate in the same animal over two consecutive estrous periods; the accuracy of this method was confirmed here.

King (1989) reported that heavier and fatter gilts had higher ovulation rates at puberty. In the present study, however, a higher ovulation rate was associated with greater fatness, but not body weight, at puberty.

It has been previously reported (reviewed by Anderson and Melampy, 1972; Brooks and Cooper, 1972; den Hartog and van Kempen, 1980) that feed restriction followed by realimentation (flushing) increases ovulation rate in swine. Because of the lack of adequate control groups, however, it never has been clear whether flushing induces superovulation or merely normalizes ovulation rate from a previously depressed level (Knott et al., 1984; Aherne and Kirkwood, 1985). From the present results the following four conclusions can be made: 1) continuous feed restriction to 50 to 65% of ad libitum (1.7 - 2 x maintenance; ARC, 1981) adversely affects ovulation rate at both first and second estrus; 2) in gilts with ad libitum access to feed (2.8 - 3.0 x maintenance), ovulation rate is higher and it increases from first to second estrus; 3) flushing (2.4 x maintenance) of gilts restrictively fed previously only increases the ovulation rate to that observed in gilts with ad libitum intake and does not stimulate superovulation; and 4) the lack of a significant correlation between the increase in ovulation and change in any production trait from first to second estrus for the LH gilts (flushing), indicates that realimentation may exert a short-term effect on ovulation rate more related to an overall improvement of metabolic status than to a dependence on changes in body weight, backfat thickness or fatness (see Booth, 1990).

E. Implications

Maximizing growth rate in replacement gilts with ad libitum access to feed may not always hasten the attainment of puberty. Indeed, excessively lean growth may delay attainment of puberty and increase mature body size and, therefore, maintenance costs. Therefore, it may be practical to manipulate growth rate by feed restriction in order to attain a target body weight at boar stimulation ($\approx$90 kg), at which minimum age ($\approx$155 d) and body weight at puberty ($\approx$97 kg) would coincide. Nutritional flushing during the first estrous cycle then could be used to normalise ovulation rate at mating at second estrus in gilts that were fed restrictively when pre-pubertal.

Table II.1. The effect of pre- and post-pubertal level of feeding on production traits[a]

Item	LL[b]	LH[b]	HH[b]	Main effect of time[c]	$P > F$	SE
Feeding level, kg/d						
47 kg to first estrus	2.0	2.0	ad lib			
n	41	43	57			
First to second estrus	2.0	2.8	ad lib			
n	34	38	43			
Age, d						
First estrus	187.0	184.5	170.1	180.5^{h}	---	---
Second estrus	208.0	205.9	191.4	201.8^{i}	.0001	SEM .19
Main effect of feeding level[c,d]	197.5^{j}	195.2^{j}	180.8^{k}	---	.0004	SEM 2.94
Feeding level x time interaction[c]	21.0	21.4	21.3	---	.7367	SED .37
Body weight, kg						
First estrus	96.6	95.7	98.9	97.1^{h}	---	---
Second estrus	104.4	107.8	114.3	108.9^{i}	.0001	SEM .33
Main effect of feeding level[c,d]	100.5	101.8	106.6	---	.0350	SEM 1.61
Feeding level x time interaction[c]	7.9^{x}	12.1^{y}	15.4^{z}	---	.0001	SED .67
Backfat thickness, mm						
First estrus	11.2	10.9	13.1	11.7^{h}	---	---
Second estrus	12.0	12.6	16.5	13.7^{i}	.0001	SEM .17
Main effect of feeding level[c,d]	11.6^{j}	11.8^{j}	14.8^{k}	---	.0001	SEM .38
Feeding level x time interaction[c]	.8^{x}	1.7^{x}	3.4^{y}	---	.0001	SED .34
Ovulation rate						
First estrus (CA)[e]	11.1	11.5	12.1	11.6^{h}	---	---
Second estrus (CL)[e]	12.0	14.0	14.7	13.5^{i}	.0001	SEM .16
Main effect of feeding level[c,d]	11.6^{j}	12.7^{k}	13.3^{k}	---	.0001	SEM .26
Feeding level x time interaction[c]	.9^{x}	2.4^{y}	2.6^{y}	---	.0009	SEM .31

[a] Least-square means for the pooled data of three consecutive years.

[b] LL = gilts that remained on a constant diet; LH = gilts that were given an increased feed allowance; HH = gilts with ad libitum access to feed.

[c] Means within a row lacking a common superscript letter differ; h, i main effect of time irrespective of feeding level; j, k main effect of feeding level irrespective of time; x,y,z feeding level x time interaction expressed by the difference between first and second estrus.

[d] Gilts (year x treatment) as error term.

[e] CA and CL = corpora albicantia and corpora lutea, respectively.

[f] Standard error of the mean (SEM) and difference (SED).

Table II.2. Correlation coefficients (r) between various production traits at puberty

Traits	No. of gilts	Mean	± SE	Age at first estrus, d	Body weight at first estrus, kg	Lifetime growth rate[a], kg/d
				r	r	r
Age at first estrus,d						
L[b]	84	185.1	2.4	---	---	---
H[c]	57	170.5	2.6	---	---	---
L + H	141	179.2	1.9	---	---	---
Body weight at first estrus, kg						
L	84	96.1	1.0	+.68**	---	---
H	57	99.1	1.8	+.76**	---	---
L + H	141	97.3	1.0	+.61**	---	---
Lifetime growth rate[a] kg/d						
L	84	.51	.01	-.57**	+.21*	---
H	57	.57	.01	-.09 NS[e]	+.57*	---
L + H	141	.53	.01	-.47**	+.41**	---
Backfat thickness at first estrus, mm						
L	59	11.1	.23	+.10 NS	+.16 NS	+.07 NS
H	34	13.4	.59	+.01 NS	+.39*	+.61**
L + H	93	12.0	.28	-.12 NS	+.28**	+.51**
Fatness[d] at first estrus, mm/kg						
L	59	.12	.01	-.32**	---	-.07 NS
H	34	.14	.01	-.49**	---	+.24*
L + H	93	.13	.01	-.48**	---	+.29**

[a] Birth to first estrus.

[b] L gilts were given ad libitum access to feed from birth to 47 kg body weight, and then 2.0 kg/d to first estrus.

[c] H gilts were given ad libitum access to feed from birth to first estrus.

[d] Backfat thickness, mm/body weight, kg

[e] NS = nonsignificant ($P > .05$);

*$P < .05$; **$P < .01$.

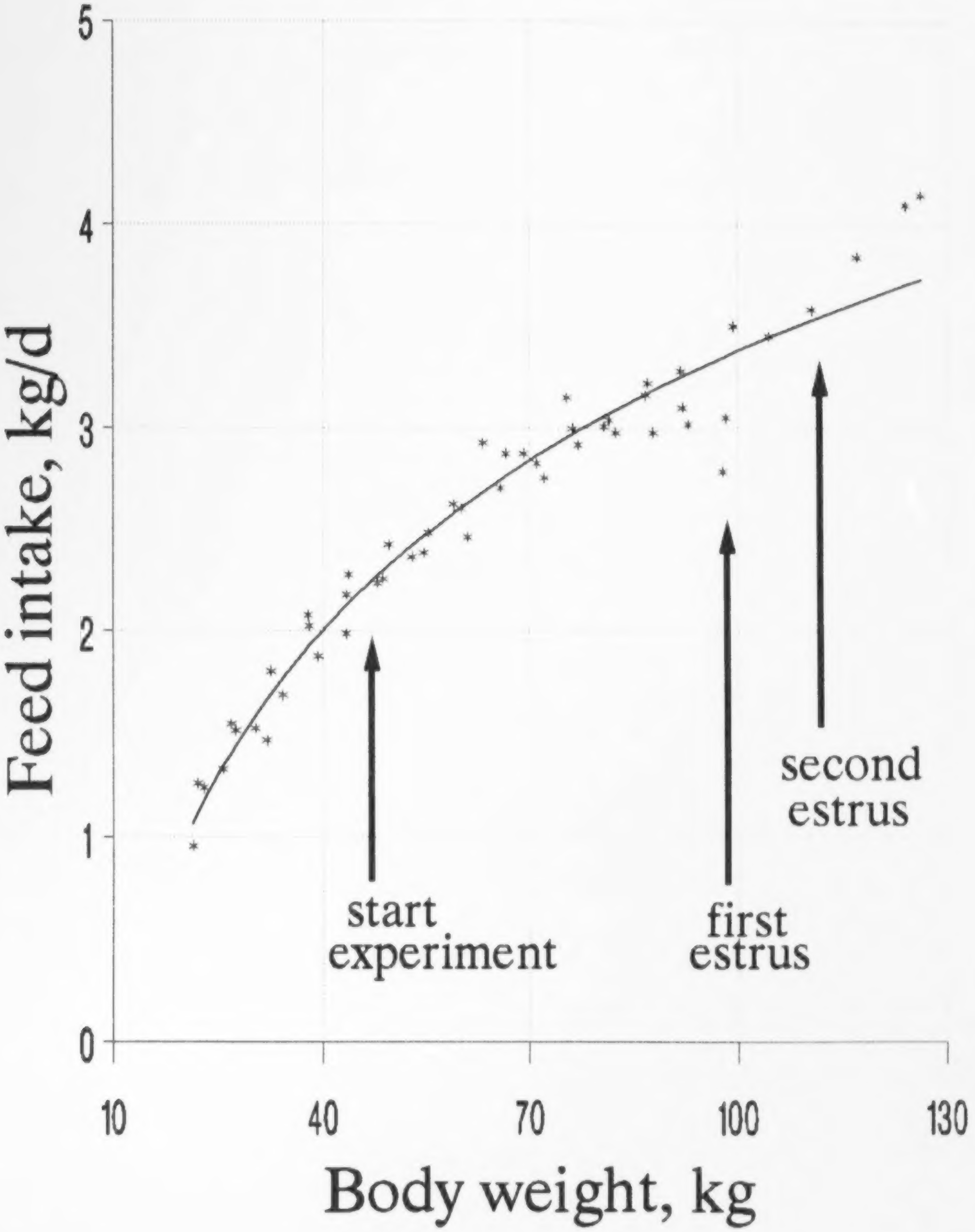

Figure II.1. Pooled daily feed intake for HH gilts in experiments 1, 2, and 3 (n = 57; $Y = .4696(\pm .1795) + .0413(\pm .0043)WT - .00012(\pm .0003)WT^2$.

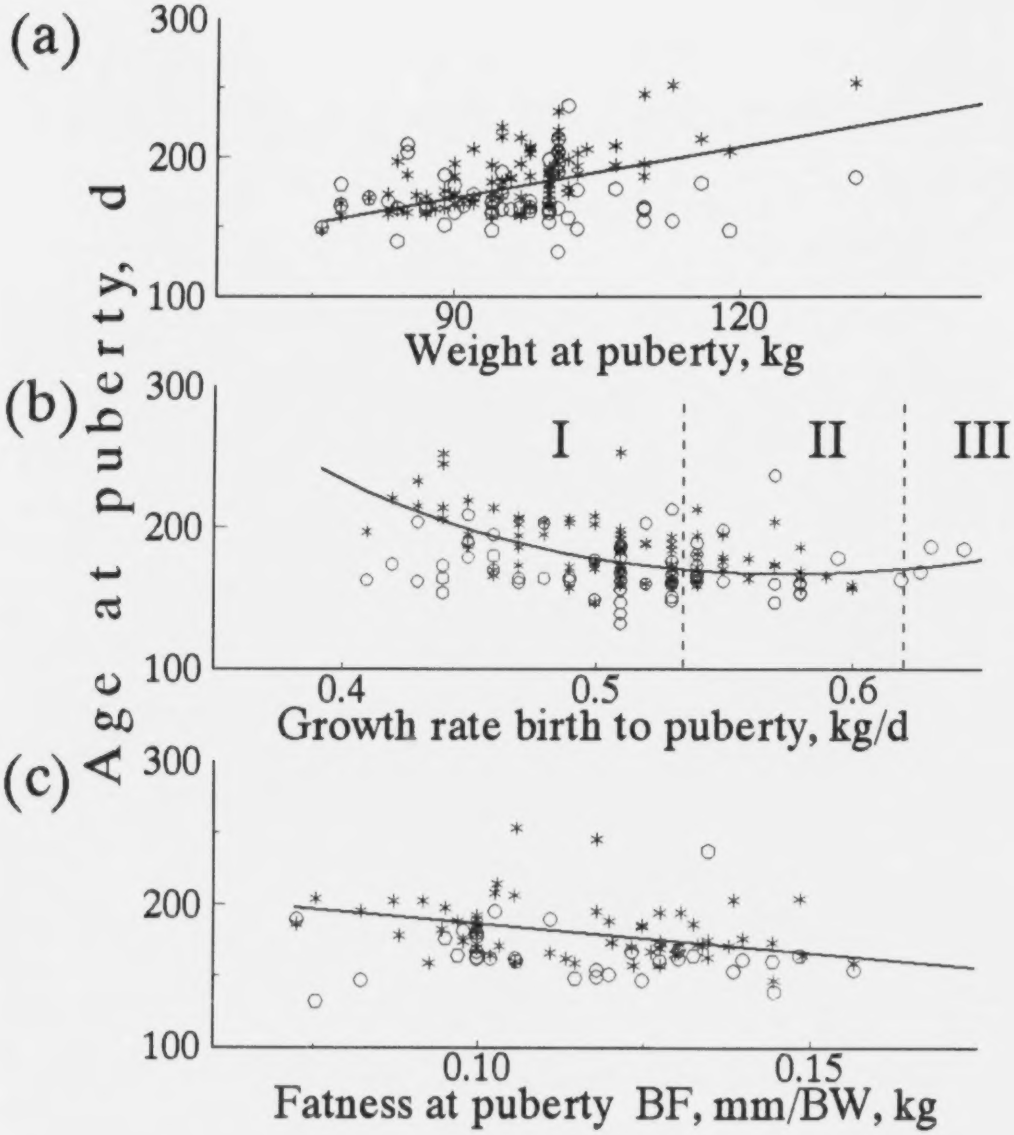

Figure II.2. Relationship between age at puberty and several production traits at puberty: (a) body weight (BW) at puberty (n = 141; Y = 62.86{± 17.74} + 1.20{± .13}BW; $P < .0001$), (b) growth rate (GR) from birth to puberty (n = 141; Y = 721.27{± 18.75} - 1835.37{± 410.07}GR + 1520.53{± 377.83}GR^2; $P < .0001$) and (c) fatness at puberty (n = 93; Y = 220.72{± 18.54} - 364.11{± 69.32}BF/BW; $P < .0001$) for the pooled data from gilts in experiments 1, 2 and 3. Feeding levels from 47 kg body weight to puberty were 2.0 kg/d(*) or ad libitum(○). Refer to the Discussion section for an explanation of the different components (I, II, and III) of the regression line in (b).

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III. ENDOCRINOLOGY OF NUTRITIONAL FLUSHING IN GILTS[2]

A. Introduction

Female prolificacy is determined genetically by the number of ova released at ovulation. Nutrition is among the environmental factors that may constrain ovulation rate. It has been long established that nutritional distress reduces lambing rate in ewes (Youatt, 1837; Coop, 1962) and litter size in pigs (Robertson et al., 1951; Christian and Nofziger, 1952; Clark et al., 1973). Nevertheless, short-term improvements in nutritional state preceding mating increases twinning rates in ewes (Nichols, 1924), and similarly, short-term, high-level feeding during the estrous cycle increases ovulation rate in gilts (Robertson et al., 1951). This phenomenon has been named "nutritional flushing" (reviewed by Anderson and Melampy, 1972; den Hartog and van Kempen, 1980; Aherne and Kirkwood, 1985 in pigs and Downing and Scaramuzzi, 1991 in sheep). In the pig, it appears that flushing increases ovulation rate but does not induce superovulation; the data presented in Chapter II corroborated the results of Self et al. (1955) in that nutritional flushing merely restored ovulation rate of gilts fed restrictively to that of contemporaries with ad libitum access to feed.

The physiological mechanisms by which an increase in feeding restores ovulation rate requires further elucidation. Since chronic, severe feed restriction impairs the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator (Armstrong and

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[2] A version of this chapter has been published. Beltranena, E., G. R. Foxcroft, F. X. Aherne and R. N. Kirkwood. 1991. Can. J. Anim. Sci. 71:1063.

Britt 1987) and a significant and rapid increase in luteinizing hormone (pLH) secretion occurs after realimentation (Flowers et al., 1988; Booth, 1990), it appears that gonadotropins mediate the restoration of ovulation rate. Whether a comparable hypothalamic-hypophyseal response occurs during realimentation after mild-restriction (i.e., flushing) however, needs clarification. Although Cox et al. (1987) have established a role for insulin as a mediator of nutritional effects on ovulation rate, in vivo evidence suggesting a role for other hormones or metabolites is limited. Roles for plasma amino acids, free fatty acids, glucose, growth hormone, thyroxine and insulin-like growth factor I (IGF-I) have been proposed mainly by extrapolation of results obtained from in vitro studies of cultured ovarian tissue (for review see Booth, 1990). The objective of the present study was to compare the endocrine status of gilts fed restrictively, flushed or given ad libitum access to feed throughout the second peri-estrus period, and thereby provide additional information of potential mediators of the ovulatory response to flush feeding.

B. Experimental Procedure

The detailed experimental procedure used in this study is described in the preceding chapter (II.B); therefore, only a brief description of the overall experimental treatments and modifications are therefore presented here.

Animals and Treatments. Forty-two gilts, progeny of Camborough dams x Canabrid sires (Pig Improvement Canada, Acme, AB) were fed a barley, wheat and soybean meal diet, formulated to provide a minimum of 16% crude protein and 12.5 MJ ME/kg

(NRC, 1979). From 45.3 SD 3.1 kg body weight and 103.4 SD 5.7 days of age until three days after the attainment of first estrus, the gilts were assigned to be fed 2.0 kg/day (L, n = 28) or had ad libitum access to feed (H, n = 14). From three days after the attainment of first estrus (puberty) until five days after the onset of second estrus, one-half of the gilts in the L group had their feed allowance increased to 2.8 kg/day (LH, n = 14), a level of realimentation calculated to be equivalent to 80% of ad libitum intake. Gilts in the H group continued to be have ad libitum access to feed (HH, n = 14). Gilts fed restrictively (LL, LH) were individually fed three meals of approximately equal size at 0630, 1130 and 1530 hours; HH gilts, in contrast, had continuous access to the diet from self-feeders.

Cannulation and Bleeding. Twelve to fourteen days after the onset of first estrus, 30 of these gilts (10 per treatment) had an indwelling catheter (i.d. 1.58 mm, o.d. 3.18 mm; Silastic, Dow Corning Corporation, Midland, MI) surgically implanted via the external jugular vein and exteriorised on the dorsal side of the neck under general anaesthesia (Fluothane, Ayerst Laboratories, Montreal, QE). These gilts were penned individually after surgery and stimulation and detection of estrus was done on an individual basis. Beginning on approximately day 14 of the first estrous cycle, until three days after the onset of second estrus, blood samples were collected at 0300, 0700, 1100, 1500, 1900 and 2300 hours into heparinized tubes. All gilts were slaughtered five to seven days after onset of their second estrus and ovulation rates were established as described in the previous chapter (II.B).

Radioimmunoassays. Plasma follicle stimulating hormone (FSH) was assayed by the homologous double antibody radioimmunoassay of Bolt et al. (1981) and Guthrie and Bolt (1983) with modifications. Porcine FSH (USDA-pFSH-PP1B; USDA Animal Hormone Program, Beltsville, MD) was used as radioiodinated (^{125}I) antigen (see Kraeling et al., 1982) and USDA-pFSH-B-1 (USDA Animal Hormone Program, Beltsville, MD) as standard (.5 to 512 ng/tube). The rabbit anti-porcine FSH serum (USDA-10-1010; USDA Animal Hormone Program, Beltsville, MD) was used at a final dilution of 1:18000. Assay sensitivity (2.5 x SD of zero standard), was 1 ng/tube. Lack of plasma interference was confirmed by demonstrating parallelism between the standard curve and increasing concentrations of a control pooled plasma (100, 200, and 300 μL/tube). Recovery of 2, 8, 18, 48, and 96 ng FSH added to 300 μL of control pooled plasma, and corrected for endogenous FSH, was 105.7%. Plasma samples were quantified in six assays with intra- and inter-assay coefficients of variation of 12.3 and 22.4%, respectively, calculated from eight to ten replicates per assay of two control-pooled plasma samples.

Plasma luteinizing hormone (pLH) was assayed using the heterologous radioimmunoassay described by Kraeling et al. (1982). Assay sensitivity was .1 ng/tube. Recovery of purified standard added to control pooled plasma averaged 103.4%. Dose response curves for different volumes of pooled plasma and increasing concentrations of hormone (.1 to 6 ng/mL; USDA-pLH-B-1; USDA Animal Hormone Program, Beltsville, MD) were parallel. The plasma samples were quantified in six assays with intra- and inter-assay coefficients of variation of 6.7 and 12.1%, respectively, calculated from six replicates per assay of a control, pooled plasma.

Plasma insulin was assayed using the homologous double antibody radioimmunoassay procedure reported by Ray et al. (1983). Purified porcine insulin (Calbiochem, La Jolla CA) was used as radioiodinated antigen and standard and IN-40 (Endocrine Science, Tarzana CA) as antiserum. Additions of .25, 2.5, 5 and 7.5 ng of hormone and 50, 100, 150 and 300 μL of pooled plasma yielded parallel inhibition curves. Plasma samples were analyzed in five assays with intra- and inter-assay coefficients of variation of 12.5 and 15.4%, respectively, calculated from four replicates each of plasma obtained from a single gilt either pre- and post-prandially.

Plasma progesterone was assayed in all 1900 hours daily samples by the method of Rawlings et al. (1977, 1980) using an antiserum raised against 4-Pregnen-11α-ol-3,20-dione hemisuccinate:BSA (N. C. Rawlings, University of Saskatchewan, Sask.). Assay sensitivity was .05 ng/tube. Intra- and inter-assay coefficients of variation for the three assays were 9.6 and 13.8%, respectively, calculated from three duplicates of pooled plasma. Inhibition curves with purified progesterone standards and various dilutions of pooled plasma were parallel. Extraction efficiency using 2 mL of double-distilled petroleum ether and 50 μL of plasma sample and determined by recovery of [1,2-^{3}H(N)]-progesterone (Dupont Canada Inc., NEN Products, Mississauga, ON) from barrow plasma, averaged 86.3%; plasma potencies were corrected for recovery.

Plasma estradiol-17β in the 0700 and 1900 hours daily samples was assayed by the method of Wettemann et al. (1972) using an antiserum raised against 1,3,5(10)-

estratrien-3,17β-diol-6-one-6CMO:BSA (N. C. Rawlings, University of Saskatchewan, Sask.). Plasma samples (1 mL) were extracted twice using 7 mL of glass-distilled diethyl ether, and extraction efficiency, as determined by the recovery of [2,4,6,7-^{3}H(N)]-17β estradiol (Dupont Canada Inc., NEN Products, Mississauga, ON) from barrow plasma averaged 91.3% in nine assays; plasma potencies were corrected for recovery. Intra- and inter-assay coefficients of variation were 11.4 and 13.9%, respectively, calculated from six replicates of pooled plasma. Initially, it was not possible to obtain parallelism between the unextracted standard curve and extracts of increasing amounts of control pooled plasma and purified estradiol-17β added to control pooled plasma. Nevertheless, when the standards were extracted from 1 mL of charcoal stripped barrow plasma, parallelism was obtained; therefore all standards were extracted from barrow plasma as described for sample tubes.

Plasma insulin-like growth factor I (IGF-I) was assayed in all 1900 hours samples corresponding to the time of individual pre-ovulatory pLH surges using a nonequilibrium, double antibody radioimmunoassay as described by Kirkwood et al. (1988), following the method of Furlanetto et al. (1977). All samples were quantified in a single assay with sensitivity and intra-assay coefficient of variation of .4 mU/mL and 2.3%, respectively.

Statistical Analyses. Statistical analyses were performed by unbalanced analysis of variance using the General Linear Models procedures (SAS, 1988) using a split-plot design that included feeding level, time and its interaction as main effects. Treatment differences were established using the error term of gilts nested within feeding level.

Differences in ovulation rate for all 42 gilts were assessed as described in the previous chapter (II.B). For all cannulated gilts, analyses for pLH, FSH, estradiol-17β and progesterone were conducted for three peri-estrous time-periods based on the time of individual pLH surges, where time zero was taken as the time of maximal pLH concentrations during the pre-ovulatory pLH surge. The pre-surge period included samples from -144 to -28 hours (day -6 to -2), the surge period from -24 to +24 hours (day -1 to +1), and the post-surge period from +28 to +72 hours (day +2 to +3). Hormonal concentrations for individual samples out of the -144 to +72 hours range were excluded from statistical analyses; uneven replication at the start of the sampling period was accounted for by the model. For the pre- and post-surge periods, sequences of individual means of six consecutive samples, representing 24-hours blocks (time), were used to assess the effect of time, and feeding level by time interaction. For the surge period, in contrast, the effect of time, and feeding level by time interaction on pLH and FSH, were assessed using individual samples taken every 4 hours. The variance of individual daily samples during the pre- and post-surge periods and the proportion of individual samples taken every 4 hours that were greater than 1 ng/mL (chi-squared; Elsaesser and Foxcroft, 1978) were also examined as indicators of the basal episodic secretion of pLH and FSH. One-way analyses of variance were conducted for the maximum pLH concentration (amplitude) and duration of the pLH surge. Duration of the pLH surge was determined as the period over which pLH concentrations were >1 ng/mL. Fisher's least-significant-difference was used to establish mean differences.

Unlike analyses for gonadotropins and steroid hormones, insulin concentrations were assessed using the daily sampling times as main effect of time. All individual samples for a given sampling time were pooled and were then related to feeding. The 0300- and 2300-hour sample concentrations were considered as estimates of pre-prandial insulin and generally corresponded to the nadir in insulin in all groups. The post-prandial response to feeding was estimated from insulin concentrations at 0700, 1100, 1500 and 1900 hours. One-way analysis of variance was conducted to establish a feeding level effect for IGF-I plasma concentrations during the pLH surge period. Fisher's least-significant-difference was used to establish mean differences.

C. Results

Feed Intake. Feed intake at initiation of the experiment was similar for the L and H groups. At puberty, however, HH gilts consumed 3.5 ± .3 kg/day or 170% more feed than the L gilts. At second estrus, feed intake for the HH gilts reached 3.8 ± .4 kg/day, equivalent to 193 and 138% that of the LL and LH gilts, respectively.

Ovulation Rates. HH and LH gilts exhibited not only a greater mean ovulation rate (main effect of feeding level irrespective of time), but also a greater increase in ovulation rate (feeding level x time interaction) between first and second estrus, compared with LL gilts (Table III.1).

Blocked cannulas from two gilts (LH, n = 1; HH, n = 1) resulted in lost plasma samples from these animals.

Changes in Plasma pLH and FSH. Changes in pLH and FSH mean plasma concentrations are shown in Figure III.1. There was no effect of feeding level, irrespective of time, on mean plasma pLH or FSH concentrations for the pre-surge, surge or post-surge periods ($P > .05$); however, a significant effect of time, irrespective of feeding level, was observed for both gonadotropins for the three peri-estrous periods ($P < .0001$; Figure III.1). During the pre-surge period, both mean pLH and FSH concentration declined. During the surge period, mean pLH concentrations increased, peaked and then decreased as is characteristic for this gonadotropin during the pre-ovulatory period. Furthermore, there was no effect of feeding level ($P > .05$) on the amplitude (LL 5.27, LH 4.72, HH 3.79 ± .41 ng/mL) or duration (LL 30.5, LH 31.2, HH 29.4 ± 3.2 hours) of the pLH pre-ovulatory surge. Mean FSH concentrations during the surge period initially increased to a peak at time 0, declined to a nadir at between +16 and +20 hours, then increased again by +24 hours. In the later post-surge period, FSH concentrations increased to a second peak by +56 hours and then declined. Variability in the episodic release of pLH during the pre-surge period was the only characteristic of gonadotropin secretion affected by feeding level. Sample variance was greater ($P < .05$) for LH and HH gilts than for LL gilts on days -6, -5 and -4 of the pre-surge period (Figure III.2, see error bars). Similarly, during the pre-surge period, LH and HH gilts had a greater ($P < .05$) proportion of samples >1 ng/mL (LL 1.1, LH 8.9, HH 10.1%)

Changes in Plasma Estradiol-17β and Progesterone. As for the gonadotropins, no effect of feeding level on plasma estradiol-17β or progesterone concentrations was observed, although a time effect was evident for both estradiol-17β and progesterone ($P < .01$). Estradiol-17β concentrations gradually increased from day -5 (17 ± 1.3 pg/mL), attaining a maximum by day -2 (39.4 ± .8 pg/mL) and returned to basal levels (7.6 ± 2.3 pg/mL) during the post-surge period. Progesterone concentrations decreased from day -1 to day 1 (5.0 ± 2.3 to 1.5 ± .7 ng/mL) and then increased with the advent of the luteal phase (28.9 ± 3.2 ng/mL by day +7).

Changes in Plasma Insulin and IGF-I. There was an effect of feeding level on overall, pre-prandial and post-prandial plasma insulin; HH gilts showed greater ($P < .05$) concentrations than LL gilts with those of LH gilts being intermediate (Figure III.3). Furthermore, an effect of time was evident also, as insulin concentrations at 0300 and 2300 hours were lower than at any other time ($P < .0001$). Plasma concentrations of IGF-I were different between the LL and HH groups ($P < .05$), with the LH group being intermediate (LH versus LL, $P = .06$; LH versus HH, $P = .07$; Figure III.4).

D. Discussion

Gonadotropin Involvement in Flushing. With the exception of possible differences in pLH pre-surge secretion, there was a lack of a significant effect of feeding level on mean plasma gonadotropins, estradiol-17β or progesterone. It has been suggested that the main effect of feeding level on ovarian activity is mediated by an alteration of plasma gonadotropin concentrations. Armstrong and Britt (1987) fed post-pubertal gilts at approximately .125 x maintenance (ARC, 1981) until they became anestrous. They noted a reduction in the frequency of pLH episodes as gilts became anestrous whereas realimentation was associated with an increased frequency of pLH episodes. As pulsatile administration of GnRH and pLH induced follicular growth and ovulation in gilts fed restrictively, the authors concluded that the long-term effect of feed restriction on ovarian function was mediated by suppression of hypothalamic release of GnRH. Furthermore, in a contemporary study by Flowers et al. (1988), increasing dietary energy from 22.6 MJ ME/day (1.4 x maintenance) to 46 MJ ME/day (2.8 x maintenance), increased ovulation rate and the number of pLH pulses but did not affect pLH pulse amplitude. Similarly, Cox et al. (1987), feeding a dietary energy level of 41.7 MJ ME/d (2.3 x maintenance) compared with 24.1 MJ ME/day (1.3 x maintenance), with or without insulin administration, augmented ovulation rate and increased pLH pulsatile release as well as basal plasma pLH and FSH concentrations. Nevertheless, in a second experiment in the same study, feeding the same high dietary energy level, accompanied by long- or short-acting insulin administration, increased ovulation rate but had no significant effect on plasma gonadotropin concentrations. As a distinct pattern of episodic LH release above a low baseline would be expected in cyclic gilts in the late luteal to early follicular phase, significant differences in the variance in pLH levels, and in the proportion of samples assayed as being >1 ng/mL, is interpreted as indirect evidence for differences in episodic pLH release in this study. If this interpretation is accepted, then these data support the concept that acute (as in flushing), as well as chronic, long-term, feed restriction followed by realimentation directly affect the hypothalamic GnRH pulse generator.

Insulin and IGF-I Involvement in Flushing. A common pattern in the three studies referred to above in the pig, and a recent report in sheep (Downing and Scaramuzzi, 1991), is that elevated plasma insulin levels were associated with an increased ovulatory response. Similar responses were seen in this study, although the differences in insulin and IGF-I concentrations between the LH and LL groups did not reach significance ($P < .1$). The relatively minor difference between the two levels of intake (2.0 and 2.8 kg/day), and the fact that the availability of gilts limited allocation of littermates across treatment, may have contributed to this lack of a significant feeding level effect.

The results of the present study are therefore consistent with previous suggestions that in the pig, increased episodic pLH secretion and elevated plasma insulin and IGF-I concentrations, acting independently or synergistically, play an important role in mediating the restoration of ovulation rate observed in response to nutritional flushing. The extent of these interactions may be determined by the severity and length of the restriction and the pattern of realimentation. Insulin appears to be the primary and, perhaps, the most important metabolic signal in each of the mechanisms proposed. First, increased plasma insulin during flush-feeding may influence both GnRH release, and hypophyseal sensitivity to GnRH, and hence gonadotropin release. Second, insulin may exert a direct effect at the ovarian level as reviewed by Booth (1990). It is pertinent to emphasize that flushing did not induce superovulation but merely restored ovulation rate in the present study (see Self et al., 1955; see Chapter II.D also). The observation of Britt et al. (1988) that insulin diminished atresia without altering the total number of follicles present, suggests that increased plasma insulin during realimentation may have also reduced the number of follicles undergoing atresia in this study, although an increased rate of follicular recruitment can not be ruled out. Third, insulin may interact with other hormones or growth factors at both the hepatic and the follicular level (see Clemmons et al., 1988; Davis, 1988). During realimentation or after growth hormone or insulin administration, synthesis of IGF-I appears to be augmented. Periods of nutritional deprivation are, in contrast, associated with decreased plasma IGF-I levels, even though plasma growth hormone levels are higher than those observed during realimentation (Buonomo and Baile, 1991). Moreover, an exogenous growth hormone challenge results in no change in plasma IGF-I concentrations under conditions of feed restriction in cattle (Elsaesser et al., 1989). Therefore, it appears that even though growth hormone is responsible for hepatic IGF-I production, hepatic tissue becomes insensitive to growth hormone due to the absence of high-affinity receptors in steers fed restrictively (Breier et al., 1987; Buonomo and Baile, 1991). An insulin threshold governs the dissociation or association of the growth hormone/IGF-I regulatory mechanism (Baxter et al., 1980).

Finally, it is suggests that the mechanisms that mediate the flushing phenomenon must be seen as responses to short-term nutritional modulation of the reproductive axis, since flushing induces a fluctuation in the energy status of the animal in the absence of major changes in body weight or composition.

E. Implications

The results of this study suggest that increased episodic pLH secretion and elevated plasma insulin and IGF-I concentrations, acting independently or synergistically, play an important role in mediating the restoration of ovulation rate observed in response to nutritional flushing.

Table III.1. The effect of pre- and post-pubertal level of feeding on ovulation rate at first and second estrus[a]

Treatment	LL[b]	LH[b]	HH[b]	Main effect		
n	14	13	14	of time[c]	$P > F$	SE[d]
Feeding level kg/d:						
45 kg to first estrus	2.0	2.0	ad lib			
First to second estrus	2.0	2.8	ad lib			
Ovulation rate:						
First estrus(CA)[e]	10.4	10.8	11.1	10.8^{u}		
Second estrus(CL)[e]	11.1	13.5	14.2	12.9^{v}	.0001	SEM .16
Main effect of feeding level[c,f]	10.7^{w}	12.1^{x}	12.7^{x}		.0078	SEM .29
Feeding level x time interaction[c]	$.7^{y}$	2.7^{z}	3.1^{z}		.0028	SED .41

[a] Least square means.

[b] LL = gilts that remained on a constant diet; LH = gilts that were given an increased feed allowance; HH = gilts with ad libitum access to feed.

[c] Means lacking a common superscript letter differ: u,v main effect of time irrespective of feeding level; w,x main effect of feeding level irrespective of time; y,z feeding level x time interaction expressed by the difference between first and second estrus.

[d] Standard error of the mean (SEM) and difference (SED).

[e] CA and CL = corpora albicantia and corpora lutea, respectively.

[f] Gilts nested within feeding level as error term.

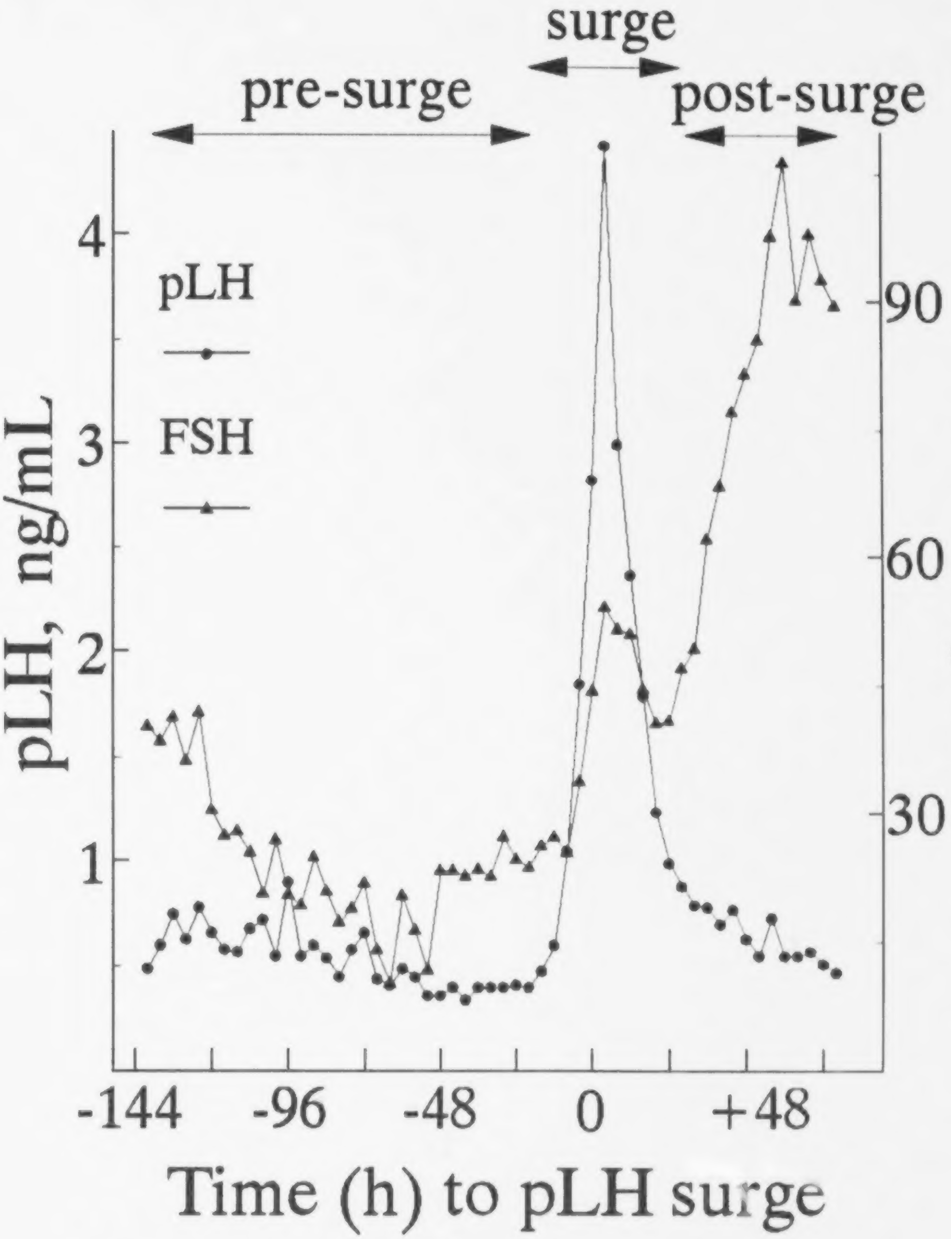

Figure III.1. Least-square means for the main effect of time irrespective of feeding level on gonadotropin plasma concentrations through the second peri-estrous period. Standard error of the mean for the pre-surge period (30 samples at 4 h intervals) were pLH ± .04, FSH ± 1.92; Surge (13 samples at 4 h intervals) pLH ± .18, FSH ± 2.83; Post-surge (12 samples at 4 h intervals) pLH ± .08, FSH ± 8.19.

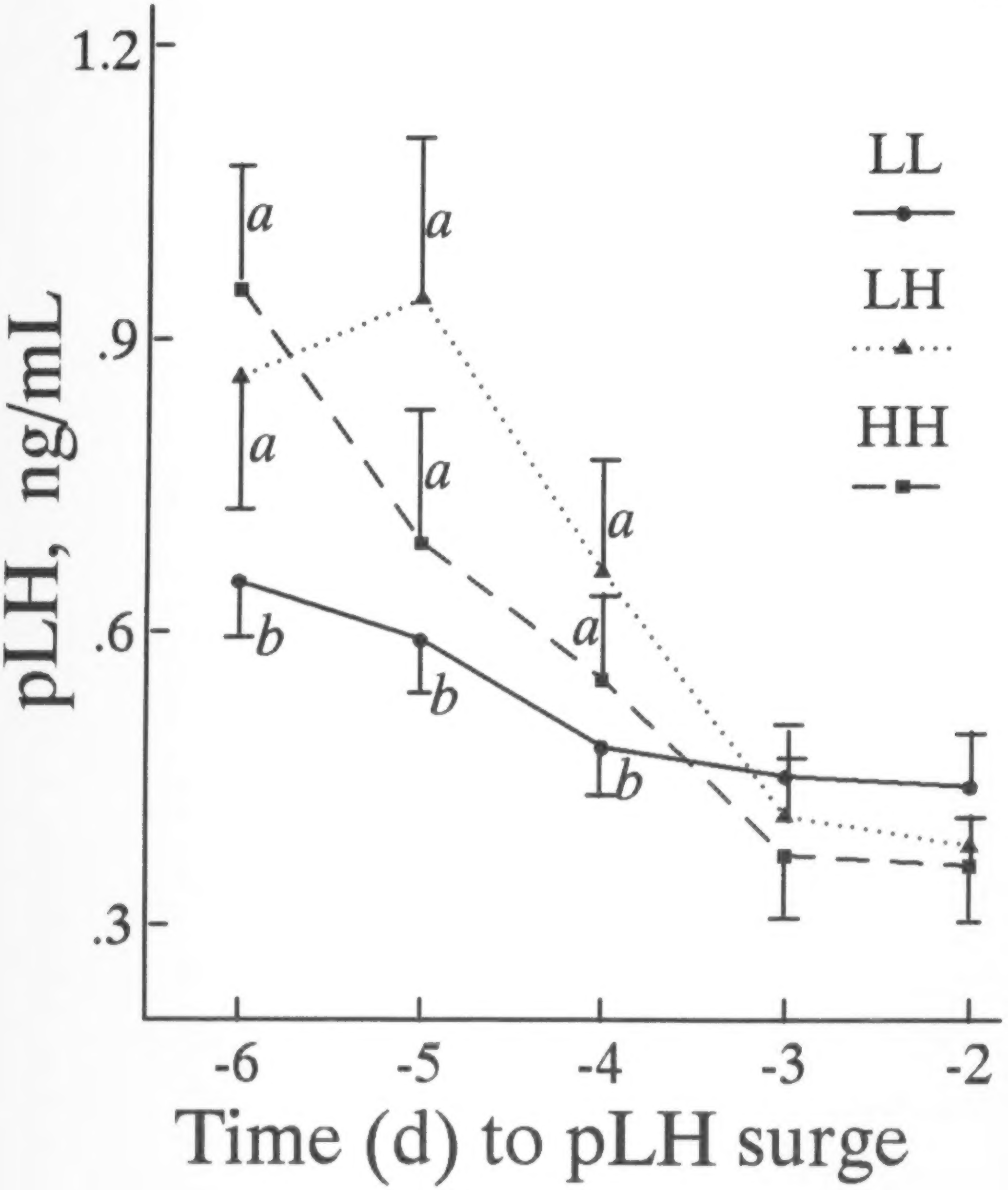

Figure III.2. Least-square means and standard errors for feeding level x time (day) for pLH during the pre-surge period. Standard error bars lacking common superscript letters differ ($P < .05$). From first to second estrus feeding levels were 2.0 kg/day (LL, n = 10), 2.8 kg/day (LH, n = 9) or ad libitum access to feed (HH, n = 9).

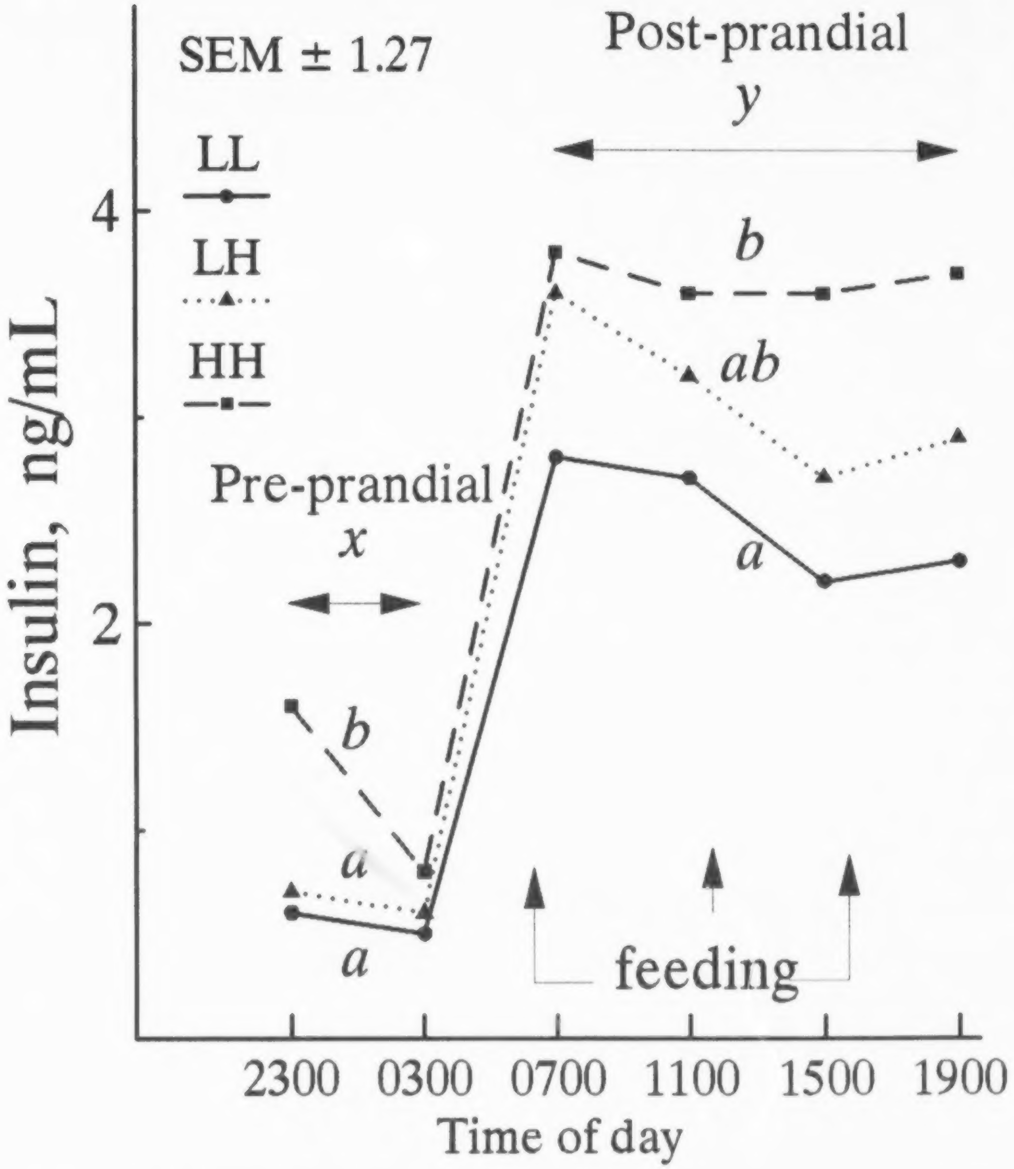

Figure III.3. Least-square means and standard error for feeding level x time of the day on pre- and post-prandial plasma insulin through the second peri-estrous period. From first to second estrus feeding levels were 2.0 kg/day (LL, n = 10), 2.8 kg/day (LH, n = 9) or ad libitum access to feed (HH, n = 9). Feeding times for gilts fed restrictively gilts (LL and (LH) were 0630, 1130 and 1530 h; Gilts in the H group had ad libitum access to feed at all times. Means lacking a common superscript letter differ ($P < .05$): a,b main effect of feeding level within a period; x,y main effect of time of the day.

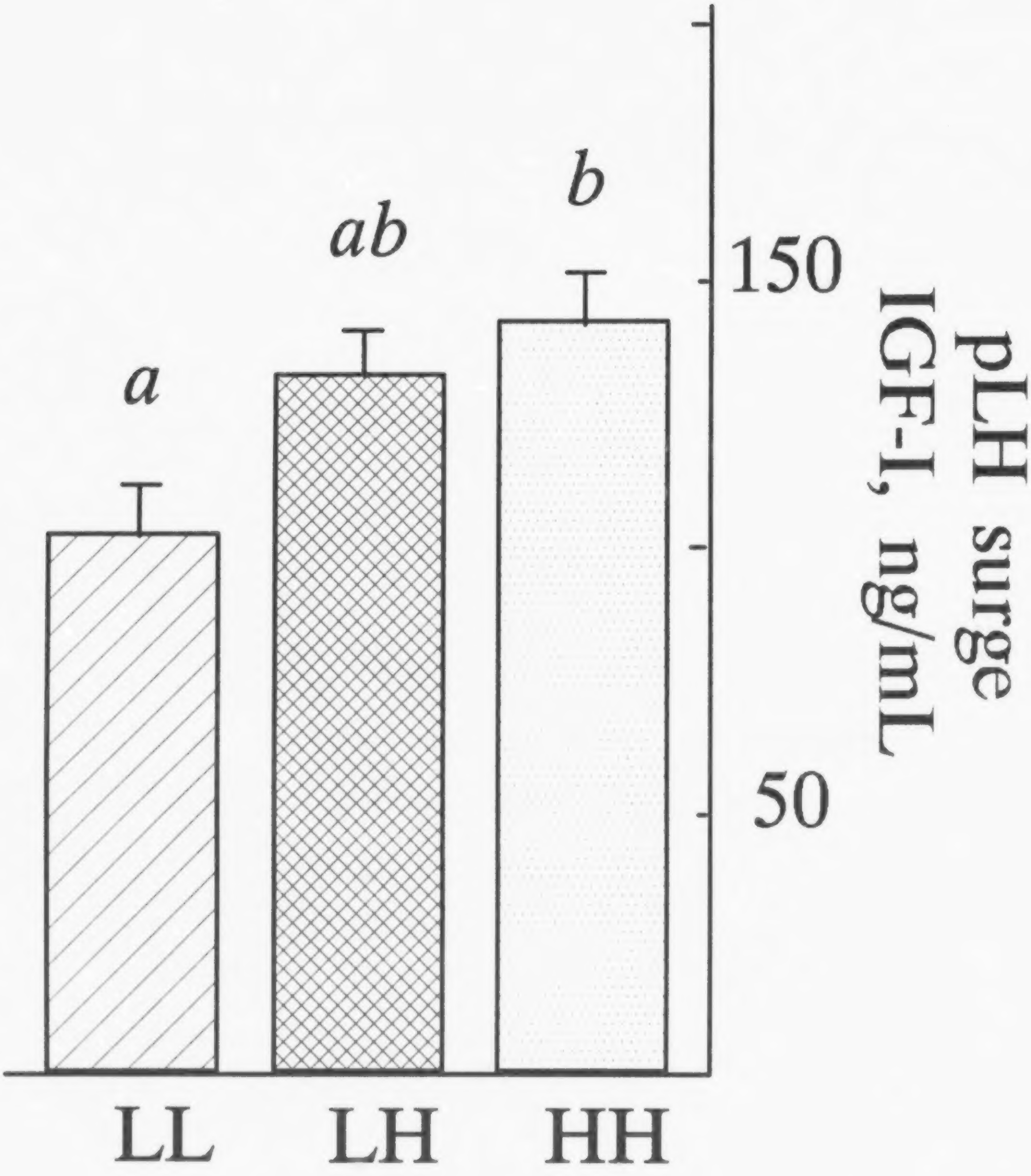

Figure III.4 Least-square means for plasma IGF-I during the pLH surge period. Means lacking a common superscript letter differ ($P < .05$): a,b main effect of feeding level. From first to second estrus feeding levels were 2.0 kg/day (LL, n = 10), 2.8 kg/day (LH, n = 9) or ad libitum access to feed (HH, n = 9).

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IV. INNATE VARIABILITY IN SEXUAL DEVELOPMENT IRRESPECTIVE OF BODY FATNESS IN GILTS[3]

A. Introduction

It has been suggested that the onset of puberty in the gilt is delayed until the attainment of a permissive level of fatness or lean:fat ratio (Kirkwood and Aherne, 1985). As modern, hybrid pigs become increasingly leaner, possible detrimental effects of extreme leanness on the attainment of puberty and subsequent reproductive performance of gilts kept as breeding replacements, may be a concern (see Johansson and Kennedy, 1983; Chapter II).

The main effects of diet composition and feeding level are reflected in the ratio of protein and fat deposition. In young pigs up to 45 kg live weight, the rate of protein deposition is linearly and positively related to feed or energy intake (Campbell et al., 1985a). Energy intake or appetite limits further increments in the rate of protein accretion (Dunkin, 1990). In contrast, a curvilinear positive relationship is apparent between maximal protein accretion rate and energy intake for pigs between 40 and 100 kg live weight. A plateau in maximal protein deposition rate is reached between 70 and 90 kg live weight (Campbell et al. 1985b), but differences exist among genotypes. Thus, increases in daily growth rate in heavier animals mostly reflects increases in fat deposition. It is, therefore, possible to use nutritional strategies to manipulate the rate of fat deposition beyond the plateau in maximal rate of protein deposition. This approach would offer the opportunity to study the effects of manipulation of body fatness on the activity of the hypothalamic-hypophyseal-ovarian axis in relation to the attainment of puberty. In the present study, therefore, the metabolic and reproductive status were characterized in littermate gilts in which different diets and dietary regimens were used with the objective of achieving maximal protein deposition but different levels of fatness.

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[3] A version of this chapter has been submitted for publication. Beltranena, E., F. X. Aherne and G. R. Foxcroft. 1992. J. Anim. Sci.

B. Experimental Procedure

Animals and Treatments. Fifty-two Camborough x Canabrid gilts (Pig Improvement Canada, Acme AB), comprising 12 sets of three to five littermates, were used. At 75 SD 1.7 days of age and 33.2 SD 4.7 kg body weight, littermate gilts with similar growth rates were allocated in a one:two or two:three ratio to have ad libitum access to either a high- (HE; n = 21) or a low-energy diet (LE; n = 31), respectively. Gilts were initially housed in groups of three according to treatment and body weight, in slatted-floor pens. Upon attainment of a mean pen body weight of 50 kg, all gilts were relocated to individual pens. Gilts in the HE group continued to have ad libitum access to the high-energy diet, whereas gilts in the LE diet were fed restrictively at approximately 80% of ad libitum. At 160 days of age, all HE and 15 LE gilts were slaughtered, while the 16 remaining LE gilts were then allowed ad libitum access to the low-energy diet until slaughtered at 175 days of age (LER).

Diets. With the intention of maximizing protein deposition rate, two ideal protein grower and finisher diets (Table IV.1) were formulated (see Stranks et al., 1988). In order to reduce or increase fat deposition, the diets were also formulated to provide different energy:lysine ratios. However, to prevent excessive energy intake, and because of the difference in energy content of the two diets (Table IV.1), the LE gilts were fed restrictively the low-energy diet. When fed restrictively at approximately 80% of ad libitum access (see Chapter II, figure II.1), the low-energy diet provided similar daily intakes of protein, amino acids, vitamins and minerals to the high-energy diet. Both diets were formulated to exceed the NRC (1988) recommendations for growing pigs. During feed restriction, LE gilts received meals of approximately equal size at 0900, 1130 and 1530 hours. All gilts were initially fed the grower diet; on attainment of an individual body weight of 75 kg, all gilts were then fed the finisher diets.

Cannulation, Bleeding and Slaughter. For the main endocrine study, 12 trios representing each litter allocated across treatment, were bled two days prior to slaughter. These gilts received indwelling jugular catheters (i.d. 1.58 mm, o.d. 3.18 mm; Silastic, Dow Corning Corporation, Midland, MI) under general anaesthesia (12 mg azaperone + 75 mg metomidate hydrochloride per 20 kg body weight, i.v.; Stresnil and Hypnodil, respectively; Jansen Pharmaceutica, Mississauga, ON). Gilts were returned immediately to their pens following surgery and individual feed intake was closely monitored during the next two days. By 1700 hours on the second day after surgery, access to feed was denied and any uneaten feed was removed. After overnight fasting, blood samples were collected at 10 min intervals from 0600 to 1500 hours and at 1530, 1600 and 1700 hours. Plasma was harvested after centrifugation of samples at 1240 x g for 15 min, and stored at -35°C until assayed. On the day of bleeding, gilts in the HE and LER groups had their pen self-feeders replaced immediately after the 0900-blood sample was collected, whereas LE gilts had their daily allowance fed at 0905, 1405 and 1730 hours. Cannulation and bleeding of littermate trios was achieved in four batches with treatments equally represented within day of bleeding. The same four batches of bled gilts together with unbled gilts were slaughtered such that treatments were similarly represented within day of slaughter.

Measurements. Individual body weight and feed intake were recorded weekly. Backfat thickness measurements were taken using the ultrasonic Krautkramer USM2 probe (Krautkramer, Cologne, Germany) in the live animal. Sites, at both sides of the midline were defined as: 1) loin, 5 centimetres off the midline, at a point aligned with the root of the curve of the last rib; 2) back, 5 centimetres off the midline and 5 centimetres in front of the stifle joint of the hind leg; and 3) grade, 7 centimetres off the midline, between the third and fourth last ribs.

During evisceration, the kidneys, the omental inter-visceral leaf lard located ventrally to the kidneys, and the head were removed and weighed separately. The hot, split, half carcasses were weighed and graded using the Hennessi grading system (Hennessi and Chong Ltd., New Zealand). Grading site was as defined for the live measurement grade, on the hot, left, half carcass (Anonymous, 1986) and measurements included carcass lean and fat depth, which were then used to calculate carcass lean yield.

Together with carcass weight, carcass lean yield was in turn used to calculate the carcass index.

The uterus and attached ovaries were also collected at slaughter and immediately placed on ice. After transport to the laboratory, the uterus was sectioned immediately posterior to the cervix, dissected from the broad ligament, fallopian tubes and ovaries, and was then weighed. The removed ovaries were then again placed on ice. Within the next hour, follicular fluid from the largest 10 follicles from each ovary, was aspirated and measured using Hamilton micro-syringes, pooled, snap-frozen and stored at -35°C until assayed.

Radioimmunoassays. Plasma samples collected every 10 min from 0600 to 1500 hours were assayed for luteinizing hormone (LH) concentrations using the homologous double antibody radioimmunoassay procedure described by Cosgrove et al. (1991). Samples were quantified in two assays with intra- and inter-assay coefficients of variation of 6.4 and 8.9%, respectively.

All plasma samples were assayed for growth hormone (GH) using the homologous radioimmunoassay procedure of Marple and Aberle (1972) with modifications: Anti-porcine growth hormone (anti-GH-202-8; D. N. Marple, Auburn University, AL) antisera and normal guinea pig serum (Calbiochem, La Jolla, CA) were used at final dilutions of 1:800000 and 1:4000, respectively. Purified porcine growth hormone (USDA-GH-I-2; USDA Animal Hormone Program, Beltsville, MD) was used as serially diluted standard (.04 - 10 ng/tube) and USDA-GH-PPI (USDA Animal Hormone Program, Beltsville, MD) as radioiodinated antigen. The bound antigen was precipitated using a locally-raised, horse, anti-guinea pig gammaglobulin (final dilution 1:25) added in a 6% polyethylene glycol solution (PEG 8000; Fisher, Nepean, ON). Addition of .15, .3, .6, 1.2 and 2.4 ng of GH standard to 200 μL of control plasma and assay of 10, 25, 50, 100, 250 and 500 μL of the control plasma pool yielded quantitative recoveries of added hormone (103.3 SD 2.8%) and parallel inhibition curves. Sensitivity, henceforth defined as 2.5 x SD of the zero standard, was .03 ng per tube. Plasma samples were quantified in two assays with intra- and inter-assay coefficients of variation of 3.4 and 5.3%, respectively.

Plasma collected pre-prandially at 0600, 0700, 0800 and 0900 hours and post-prandially at 0920, 0940, 1000, 1030, 1100, 1200, 1300, 1400, 1420, 1440, 1500, 1530, 1600 and 1700 hours was assayed for insulin and insulin-like growth factor I (IGF-I) concentrations. Plasma insulin concentrations were determined using a homologous double antibody radioimmunoassay based on the methodology described by De Boer and Kennelly (1989). Porcine insulin (Calbiochem, La Jolla, CA) was used as radioiodinated antigen and standard. Tracer was added in assay buffer containing normal guinea pig serum at a 1:1050 final dilution. The guinea pig, anti-pig first antibody (Calbiochem, La Jolla, CA) and the goat, anti-guinea pig gamma globulin (Calbiochem, La Jolla, CA) were used at final dilutions of 1:168000 and 1:245, respectively. Specificity and accuracy of the assay were demonstrated by obtaining parallelism between additions of 12.5, 25, 50 and 100 μL of a control pooled plasma and the standard curve, and quantitative recovery (99.6 SD 3.1%) of additions of .075, .15, .3 and .6 ng insulin to 200 μL of control pooled plasma. Sensitivity of the assay was .01 ng per tube. Plasma samples were analyzed in one assay with intra-assay coefficient of variation of 5.6%.

Insulin-like growth factor I concentrations were assayed using the homologous double antibody radioimmunoassay procedure described by Glimm et al. (1988) with modifications: Individual 100 μL aliquots of plasma or 50 μL aliquots of follicular fluid pooled from the largest 10 follicles were extracted by vortexing with 3 and 1.5 mL, respectively, of acid-ethanol (12.5 HCl (2.2 M), 87.5 ethanol {v/v}), and incubated at room temperature for 20 hours before centrifugation at 1522 x g for 30 minutes at 4°C. Supernatant aliquots of 200 μL were neutralized with 800 μL of Tris-base buffer (.0855 M; Sigma Chemical Co., St. Louis, MO), stored at 4°C and assayed within 72 hours. The antiserum anti-hIGF-I (USDA Animal Hormone Program, Beltsville, MD) was used at a final dilution of 1:25164. Normal rabbit serum obtained locally and goat, anti-rabbit gamma-globulin (Calbiochem, La Jolla, CA) were used at final dilutions of 1:3150 and 1:980, respectively. Sensitivity of the assay was .004 ng per tube. Additions of 25, 50, 100, 200 and 400 μL of plasma and 25, 50, 100, 200 and 400 μL of a pool of control follicular fluid produced inhibition curves parallel to the standards, confirming specificity; additions of .014, .028, .057 and .114 ng of IGF-I added to pools of control plasma and follicular fluid prior to extraction yielded 97.4 SD 4.5% recovery, conferring accuracy. All plasma samples were quantified in two assays with intra- and inter-assay coefficients of variations of 5.7 and 7.3%, respectively. All follicular fluid samples were quantified in one assay with an intra-assay coefficient of variation of 3.4%.

Non-esterified fatty acids (NEFA) concentrations were quantified in all pre- and post-prandial plasma samples by an enzymatic colorimetric method (Wako Pure Chemical Industries, Ltd. Osaka, Japan). Plasma samples were analyzed in 3 assays with intra- and inter-assay coefficients of variation of 4.1 and 6.9%, respectively.

Plasma samples collected at 0700 and 1030 hours were assayed for creatinine and urea nitrogen employing enzymatic colorimetric methods (Sigma Chemical Co. St. Louis, MI). Samples were quantified in single assays with intra-assay coefficients of variation of 3.9 and 4.9%, for creatinine and urea nitrogen, respectively.

Individual pooled plasma, prepared from equal volumes of plasma collected at 0700, 1100 and 1400 hours, was assayed for estradiol-17β concentrations using the homologous double antibody radioimmunoassay described by Webb et al. (1985) with minor modifications: Aliquots of 200 μL of plasma sample and 100 μL of standard estradiol-17β (.2 - 400 pg/tube; Sigma Chemical Co. St. Louis, MI) were extracted with 5 mL of diethyl ether (BDH Inc., Toronto, ON) by vortexing for 8 minutes using a multi-tube vortexer (American Scientific Products, McGaw Park, IL). The aqueous phase was frozen by immersion in a methanol-liquid N_2 bath and supernatants were decanted into assay tubes and vacuum-dried (Speedvac concentrator SVC200H, Farmingdale, NY). The ^{125}I-17β-estradiol-11α-TME and anti-estradiol-17β [R 48] antiserum (R. Webb, AFRC I.A.P.G.R. Roslin, Scotland) used at a final dilution of 1:2000000, were then added in assay buffer and tubes were incubated for a 2-hour period. Normal rabbit serum obtained locally and goat, anti-rabbit gamma-globulin (Calbiochem, La Jolla, CA) were used at final dilutions of 1:1250 and 1:3000, respectively. Specificity of the assay was demonstrated by obtaining parallelism between extractions of 50, 100, 200 and 400 μL of a control plasma and the standard curve; extraction efficiency was estimated by additions of 100 μL ^{3}H-estradiol (30,000 dpm; Dupont Canada Inc., NEN Products, Mississauga, ON) to duplicate control plasma tubes included with each batch of samples vortexed, and gave an overall mean recovery of 93.1%. Sensitivity of the assay was .4 pg per tube. Plasma samples were analyzed in one assay with an intra-assay coefficient of variation of 8.5%.

Individual pooled follicular fluid samples from the largest 10 follicles were assayed for estradiol-17β concentrations as described in Chapter III.B, and validated for assay of follicular fluid as follows: Aliquots of 100 μL of assay buffer-diluted follicular fluid and estradiol-17β standard (.0008 - 1.6 ng/tube; Calbiochem, La Jolla, CA) were added ^{3}H-estradiol-17β (Dupont Canada Inc., NEN Products, Mississauga, ON) and anti-estradiol-17β (rabbit A11; N. C. Rawlings, Western Coll. of Veterinary Medicine, University of Saskatchewan, SA) at a final dilution 1:600000 and were incubated for 24 hours at 4°C. Free ligand was adsorbed by addition of a cold, equilibrated suspension of .1 mg dextran (Calbiochem, La Jolla, CA) and 1 mg activated charcoal (Norit-A; Calbiochem, La Jolla, CA) per tube, vortexed and immediately centrifugated at 1057 x g for 10 minutes at 4°C. Supernatants were decanted in vials containing liquid scintillation fluid (ICN Biomedicals, Inc. Irvine, CA), mixed thoroughly, and counted. Specificity of the assay was demonstrated by obtaining parallelism between 100 μL additions of 1:50, 1:100, 1:200, 1:400 and 1:800 dilutions of a pooled, control follicular fluid and the standard curve; accuracy, using direct assay without extraction, was determined from quantitative recovery (102 SD 2.8%) of 3.13, 6.25, 12.5, 25 and 50 pg ^{3}H-estradiol added to pooled, control follicular fluid. Sensitivity of the assay was 3.1 pg per tube. Plasma samples were analyzed in one assay with intra-assay coefficient of variation of 5.9%.

Statistical Analyses. Statistical analyses were performed using the General Linear Models procedures of SAS (1990). Treatment differences in live body weight, backfat thickness and daily energy and protein intake prior to slaughter and carcass measurements after slaughter, were assessed by unbalanced analysis of variance; for these, live body weight prior to slaughter and carcass weight were initially considered as covariates, but as they proved nonsignificant ($P > .05$), they were removed from the model. In order to quantify the episodic secretion of LH and GH, data were subjected to cluster (Veldhuis and Johnson, 1986) and sliding window (Shaw and Foxcroft, 1985) analyses, using visual appraisal of individual profiles to confirm episodic secretion (Foxcroft et al., 1988). Treatment and litter differences for episodic characteristics of LH and GH, reproductive tract weight, total follicular fluid volume, plasma and follicular fluid estradiol-17β, were also assessed by unbalanced analysis of variance. Treatment differences involving a change over time, for body weight, backfat thickness, energy intake, insulin, IGF-I, NEFA, creatinine and urea nitrogen, were assessed using time, litter and their two-way interactions as subplot effects. The variance of treatment x litter was used as the error term to test treatment effects. Further statistical analyses of insulin, IGF-I, and NEFA plasma concentrations were conducted with respect to time periods defined in relation to the feeding schedule of the LE gilts on the day of bleeding. Bleeding or slaughter batch was also initially considered as an additional effect, but as it proved nonsignificant, it was removed from the model. Comparisons between treatment means were performed by Fisher's least-significant difference test. Associations between characteristics of reproductive development and episodic LH secretion were explored using linear regression and Pearson correlation analyses.

C. Results

Animals. Two gilts from the HE group were withdrawn from the experiment during the growing period, one because of leg weakness, the other because of unsatisfactory growth rate associated with respiratory illness. Furthermore, all gilts in one litter were excluded entirely from statistical analyses of parameters associated with reproductive development, as post-mortem examination revealed the presence of corpora lutea in four of the five gilts in the litter, indicating that these gilts had already attained puberty, irrespective of treatment.

Live Weight, Backfat Thickness and Energy Intake. As shown in Table IV.2, HE gilts were, on average, 10.7 kg heavier, 3.5 mm fatter and consumed 14.9 MJ more digestible energy per day than LE gilts by 160 days of age. Gilts in the LER group were 8.2 kg heavier, 2.2 mm fatter and consumed 22 MJ more digestible energy per day by 175 days of age than LE gilts at 160 days of age. Treatment x time interactions (slope) were significant for body weight and backfat thickness from 115 days of age onwards (Figure IV.1; top, $P < .005$ and middle, $P < .0001$, respectively), and for energy intake from 100 days of age onwards (Figure IV.1; bottom, $P < .0001$).

Carcass Measurements. As shown in Table IV.2, LE gilts had lower live body weight prior to, and lower carcass weight following, slaughter than HE or LER gilts. Similarly, LE gilts had lower carcass fat depth than either HE or LER gilts; carcass lean depth was not different among groups ($P > .05$). Furthermore, there were no significant effects of dietary regimen on carcass yield or index ($P > .05$; Table IV.2). Gilts in the HE group had greater leaf lard than those in the LE group, with those in the LER group being intermediate (Table IV.2).

Reproductive Status. The number of LH episodes could not be clearly established in six gilts, using either cluster analysis or visual appraisal of episodes. In these gilts the frequency of episodic secretion was high and associated with an elevated LH baseline (Figure IV.2); moreover, these gilts had the heaviest reproductive tracts and the largest follicular fluid volume. Treatment means, maximums, minimums and profile areas for all gilts were therefore established by sliding window analysis and are presented in Table IV.3. For gilts categorized as having a low episodic frequency, treatment means for basal and episodic LH secretion, as established by cluster analysis, are presented in Table IV.4. Basal and episodic LH characteristics were not affected by treatment or litter ($P > .05$) either overall or in gilts categorized as having a low episodic LH frequency. Treatment means for reproductive tract weight, total follicular volume, follicular fluid and plasma estradiol-17β concentrations for all gilts are presented in Table IV.5. As for basal and episodic LH secretion, treatment or litter had no effect on these parameters of reproductive tract and ovarian development ($P > .05$). Overall and within each treatment, however, there were significant correlations ($P < .05$) between reproductive tract weight and total follicular volume, and several indicators of basal and episodic LH secretion. As shown in Figure IV.3, reproductive tract weight and total follicular fluid volume increased as episodic frequency and basal LH concentration increased, but episodic amplitude decreased.

Metabolic Status. Applying the same methods of analysis as used for LH, revealed no effect of treatment or litter on any indicator of basal or episodic GH secretion (Table IV.6, sliding window analysis; Table IV.7, cluster analysis).

Plasma concentrations of insulin, IGF-I and NEFA for HE, LE and LER gilts are presented in Figure IV.4. Pre-prandial plasma insulin was not different among treatment groups ($P < .3926$; Figure IV.4, top). Insulin concentrations increased sharply after resumption of feeding at 0900 hours, reached a maximum 40 to 60 minutes later, and then declined in all treatment groups; plasma insulin increased again after feeding of the LE gilts at 1400 hours. Gilts in the LER group showed greater and more sustained insulin concentrations than either LE or HE gilts during the post-prandial period following feeding at 0900 hours ($P < .0313$).

Pre- and post-prandial IGF-I concentrations (Figure IV.4, middle) were not different among groups for any period ($P > .05$).

Plasma NEFA concentrations decreased after the 0900-feeding in all groups, yet were greater in HE than in LE or LER gilts for the pre- and both post-prandial periods following the 0900- and 1400-meals ($P < .0005$, $P < .0003$, $P < .0004$, respectively;

Figure IV.4, bottom). Treatment x time interactions for each period for insulin, IGF-I and NEFA were nonsignificant ($P > .05$).

Despite the higher protein intake in the LER gilts, plasma urea nitrogen concentrations were not different among treatment groups (HE, 69.42 ± 5.27; LE, 74.18 ± 5.27; LER, 77.13 ± 5.27 mg/dL; $P > .1130$). There were no significant effects of time irrespective of treatment or treatment x time interactions ($P > .05$).

Plasma creatinine concentrations were greater in HE than in LER gilts, but not different from those of LE gilts (HE, 1.59 ± .04; LE, 1.49 ± .04; LER, 1.38 ± .04 mg/dL; $P < .0080$). The effects of time irrespective of treatment, and treatment x time interaction were again, nonsignificant for creatinine concentrations ($P > .05$).

D. Discussion

Effectiveness of the Dietary Regimens. The measurements of backfat thickness prior to slaughter, and the carcass fat depth measurements following slaughter, suggest that the dietary regimens imposed in the present study were effective in inducing disparate levels of body fatness between HE and LE gilts by 160 days of age. The carcass lean depth measurements suggest that both HE and LE gilts achieved similar levels of lean deposition and re-feeding of LE gilts (LER) did not induce further increases in carcass lean suggesting that similar levels of protein deposition were probably achieved.

Effect on Hormonal Metabolic Status. The lack of a significant effect on plasma urea nitrogen concentrations among treatments groups suggests, first, that the supply of dietary protein was probably adequate to attain similar levels of body protein accretion;

and second, that the level of feed restriction imposed on the LE gilts did not cause significant body protein catabolism; the latter would have resulted in increased plasma urea nitrogen as a consequence of amino acid deamination. The similarity in plasma creatinine and NEFA concentrations between the LE and LER gilts, also suggests that neither skeletal muscle, nor fat, were mobilized to a great extent by the LE gilts; skeletal muscle and fat mobilization could have occurred in order to compensate for the lack of dietary energy supply. The greater circulating plasma creatinine and NEFA concentrations observed in the HE gilts, are likely associated with the higher dietary fat intake, rather than with increased body tissue wasting; these had no evident effect on basal or episodic LH secretion, nor on reproductive tract weight or follicular development.

With respect to metabolic hormonal status, the greater and more sustained insulin concentrations observed in LER gilts in comparison to HE and LE gilts are likely a consequence of realimentation. A comparable trend in gilts realimented during the first estrous cycle following feed restriction during finishing period was previously observed (Chapter II). Elevated circulating insulin concentrations have been suggested as the metabolic signal mediating the increase in ovulation rate in response to increases in dietary energy intake (Cox et al., 1987; Britt et al., 1988). In the present study, it appears that elevated post-prandial insulin was of no physiological significance, as basal and episodic LH secretion, reproductive tract weight and follicular development appeared to have been unaffected. Furthermore, level of fatness, as altered by dietary regimen, had no effect on basal or episodic GH secretion.

Effect of Fatness on Reproductive Status. Previous reports have shown either a negative linear correlation (den Hartog and Noordewier, 1984; Burnett et al., 1988; Eliasson et al., 1990; Young et al., 1990; see Chapter II.D also), or no correlation (Reutzel and Sumption, 1968; Cunningham et al., 1974; Friend et al., 1981; Hutchens et al., 1981; Mabry et al., 1985), between backfat thickness and age at attainment of puberty in the gilt. In establishing these associations, however, the effects of body fatness were not separated from the confounding effect of body weight or growth rate. In other studies this relationship was further confounded by boar stimulation (Burnett et al., 1988; see Chapter II.D also). Using the model described for the present study, the possible effects of fatness on reproductive development were successfully segregated from those of lean tissue growth. Moreover, the gilts in the present study were not exposed to boars.

Using the model described for the present study, the data show that, in the presence of similar levels of carcass lean deposition, there was no apparent effect of a reduced compared with an increased level of carcass fat on basal and episodic LH secretion, follicular development, reproductive tract weight, and plasma or follicular estradiol-17β concentrations. Similarly, de Ridder et al. (1990) have also reported that there was no relationship between body fat mass and plasma gonadotropins and total plasma sex steroid concentrations in early pubertal girls. Body fat distribution, rather than body fat mass, was reciprocally related to the fraction of sex steroids not bound to sex hormone-binding globulins; the free steroid fractions were considered the fractions available for biological activity. In that study, de Ridder et al. (1990) observed that girls with fat localized in the hips, had the highest levels of sex steroids and gonadotropins. In the present study, leaf lard weight was used as an indicator of intervisceral fatness. Because of its close proximity to the ovaries and reproductive tract, it was thought it could influence sex-steroid metabolism. However, no relationship was established between leaf lard weight and basal or episodic LH secretion and plasma or follicular fluid estradiol-17β.

Using a different model, it has been possible to gain an understanding of the threshold in corporal fat that is permissive of the attainment of puberty (E. Beltranena, unpublished data). In 85 d-old gilts, fed restrictively to maintain body weight at 50 kg until 270 days of age, backfat reached a constant minimum thickness of 5 mm by 120 days of age. Following realimentation, attainment of puberty occurred after approximately a 20 kg gain in body weight, but only a further 1 mm gain in backfat thickness. Thus, the fatness threshold for the attainment of puberty appears to be equivalent tc 6 mm of backfat thickness. That and the present study, are of great significance because they indicate that, within a wide fatness range (equivalent to 6 to 16 mm in backfat thickness), body fatness can be independent of sexual development. The results of these two studies also contradict previous suggestions that the onset of puberty in the gilt may be delayed due to the need to attain a permissive level of fatness or lean:fat ratio (Kirkwood and Aherne, 1985; Kirkwood et al., 1987). Young et al. (1990) using a large number of gilts allowed access to feed ad libitum, has recently shown that backfat thickness and fatness, estimated as backfat thickness/body weight, show a normal rather than a skewed distribution at puberty. Thus, the results of these studies and those of Young et al. (1990), indicate that commercially grown gilts have fatness levels well in excess of that threshold required for the attainment of puberty.

Innate Variability in Sexual Development Irrespective of Body Fatness and(or) Dietary Regimen. Of particular relevance in the present study, are the significant correlations between reproductive tract weight and follicular fluid volume and indicators of basal and episodic LH secretion. Gilts categorized as having a high episodic LH frequency, also had the heaviest reproductive tract weight and largest follicular fluid volume. Moreover, in gilts categorized as having a low episodic LH frequency, reproductive tract weight and follicular fluid volume increased as episodic frequency and basal LH concentration increased, but episodic amplitude decreased. Not withstanding an effect of treatment, these data clearly illustrate that the gilts had achieved a level of reproductive development advanced enough to respond to endogenous gonadotropic stimulation. Under the controlled conditions of the present experiment, these results underline the presence of innate genetic variability in the rate of sexual maturation and that this is associated with inherent differences in the pattern of gonadotropin secretion. This innate variability in the rate of sexual maturation is also reflected from the normal distribution in age at puberty reported by Young et al. (1990). In this context, it should be noted that one litter had to be excluded from statistical analyses of characteristics associated with reproductive development as four of the five gilts had attained puberty by time of slaughter. This litter group may be assumed to represent the lower limit in sexual precocity in this population of gilts.

E. Implications

The results of the present study suggest that differences in the level of fat deposition, in the presence of similar levels of lean content, do not influence sexual development in the gilt. Not withstanding differences in metabolic state, innate variability in episodic and basal LH secretion was associated with differences in follicular and reproductive tract development.

Thus, it can be concluded that under contemporary husbandry conditions, the fatness of market weight gilts is unlikely to limit reproductive development if the gilts are kept as breeding replacements.

Table IV.1. Low-energy (LE) and high-energy (HE) diets[a]

Item, kg/ton	LE Grower[b]	HE Grower[b]	LE Finisher[b]	HE Finisher[b]
Wheat	400	400	420	420
Barley	309	328	350	360
Soybean	240	150	180	100
Tallow	---	80	---	80
Limestone	13	11	13	11
Ca_2PO_4	16	12	16	12
Salt	5	4	5	4
Vitamins & minerals[c]	12.5	10	12.5	10
Lysine	3.4	3.4	2.5	2.5
Threonine	1.2	1.2	.5	.5
Methionine	---	---	.5	---
Calculated analysis :				
Energy, MJ DE/kg	13.2	14.9	13.1	14.9
Crude Protein, %	20.2	16.2	18.1	14.5
Ideal Protein, %	16.3	13.0	13.3	10.2
Lysine, %	1.14	.91	.93	.72
Methionine + cystine, %	.63	.52	.63	.48
Threonine	.69	.56	.56	.43
Lysine, g/MJ DE	.86	.61	.71	.48
Calcium, %	.88	.71	.88	.70
Phosphorus, %	.74	.60	.74	.59

[a] Approximately 90% dry matter.

[b] Gilts in the HE group had ad libitum access to the high-energy diet until slaughter at ≈ 160 d of age. Gilts in the LE group were fed restrictively after 50 kg (≈ 80% of ad libitum) the low-energy diet until slaughtered at ≈ 160 d of age, or were then allowed ad libitum access to the low-energy diet until slaughtered at ≈ 175 d of age (LER).

[c] Premix provided the following per kg of diet: Vit. A 5000 IU, Vit. D 500 IU, choline 300 mg, D-calcium pantothenate 25 mg, riboflavin 12 mg, niacin 45 mg, Vit. B_{12} 30 μg, Vit. E 40 IU, iodine .2 mg, manganese 12 mg, ethoxyquin 2.5 mg, copper 20 mg, zinc 120 mg, selenium .1 mg, iron 150 mg.

Table IV.2. Effects of dietary regimen on growth traits and carcass measurements of pre-pubertal gilts[a]

Item	HE[b]	LE[b]	LER[b]	*P* > *F*[c]
n	19	15	16	
Energy intake prior to slaughter, MJ DE/d	$52.1^x \pm .83$	$37.2^y \pm .93$	$58.2^x \pm .89$	.0054
Protein intake prior to slaughter, g/d (last week)	$507.0^x \pm 12.32$	$513.9^x \pm 4.33$	$704.1^y \pm 19.87$	.0002
Age at slaughter, d	$160.5^x \pm .42$	$161.2^x \pm .51$	$175.6^y \pm .52$	.0016
Live weight prior to slaughter, kg	$100.2^x \pm 1.36$	$92.2^y \pm 1.53$	$99.4^x \pm 1.41$	.0012
Backfat thickness prior to slaughter, mm	$14.2^x \pm .04$	$10.7^y \pm .05$	$12.9^x \pm .05$	.0491
Carcass measurements :				
Weight, kg	$79.7^x \pm 1.14$	$72.6^y \pm 1.34$	$77.8^x \pm 1.28$	.0016
Lean, mm[d]	44.5 ± 1.39	42.1 ± 1.52	44.7 ± 1.43	.3683
Fat, mm[d]	$16.8^x \pm .89$	$13.8^y \pm .95$	$16.5^x \pm .96$	.0464
Yield, %[d]	$50.9 \pm .31$	$51.8 \pm .42$	$51.0 \pm .35$	.1644
Index[e]	107.0 ± 1.5	104.4 ± 1.7	107.0 ± 1.6	.4614
Leaf lard, kg	$1.3^x \pm .09$	$.9^{yz} \pm .13$	$1.1^{xz} \pm .12$	.0275

[a] Least square means ± SEM.

[b] Gilts in the HE group had ad libitum access to the high-energy diet until slaughter at ≈ 160 d of age. Gilts in the LE group were fed restrictively after 50 kg (≈ 80% of ad libitum) the low-energy diet until slaughtered at ≈ 160 d of age, or were then allowed ad libitum access to the low-energy diet until slaughtered at ≈ 175 d of age (LER).

[c] Means within a row lacking a common superscript letter differ.

[d] Hennessi grading system.

[e] Canadian Hog Carcass Grading/Settlement System.

Table IV.3. Effects of dietary regimen on characteristics of episodic LH secretion in pre-pubertal gilts (all gilts) as established using sliding window analysis[a].

Item	HE[b]	LE[b]	LER[b]		
n	11	11	11	SEM	$P > F$[c]
Profile mean, ng/mL	.48	.48	.47	± .05	.9920
Profile maximum, ng/mL	.68	.68	.66	± .07	.9789
Profile minimum, ng/mL	.33	.33	.33	± .03	.9940
Profile mean area, ng/(mL·60 min)	.42	.42	.42	± .05	.9995

[a] Sliding Window analysis parameters: window size, 1 h; sampling frequency, 6/h; substraction for baseline, .05 ng/mL.

[b] Gilts in the HE group had ad libitum access to the high-energy diet until slaughter at ≈160 days of age. Gilts in the LE group were fed restrictively after 50 kg (≈80% of ad libitum) the low-energy diet until slaughtered at ≈160 days of age, or were then allowed ad libitum access to the low-energy diet until slaughtered at ≈175 days of age (LER).

[c] Means within a row lacking a common superscript letter differ.

Table IV.4. Effects of dietary regimen on characteristics of LH secretion in pre-pubertal gilts (low-frequency only) as established using cluster analysis[a,b,c]

Item	HE[d]	LE[d]	LER[d]	
n	9	10	8	$P > F$[e]
Profile mean concentration, ng/mL	.48 ± .05	.46 ± .05	.46 ± .06	.9773
Profile total area, ng/(mL • 360 min)	256.53 ± 30.49	250.03 ± 28.92	247.09 ± 34.57	.9772
Episodic characteristics :				
Peaks, #/550 min	3.56 ± .68	4.00 ± .65	4.86 ± .78	.4608
Interval between peaks, min	132.51 ± 18.05	109.33 ± 17.12	114.38 ± 22.11	.6365
Mean width, min	91.76 ± 9.61	67.23 ± 9.12	68.03 ± 10.90	.1670
Mean height, ng/mL	1.27 ± .12	0.99 ± .11	.96 ± .13	.1496
Maximum height/pre-peak nadir, %	508.10 ± 45.75	382.90 ± 43.41	437.58 ± 51.88	.1487
Mean height/pre-peak nadir, %	187.59 ± 8.66	173.86 ± 8.22	177.81 ± 9.82	.5136
Mean area, ng/(mL • width)	16.92 ± 2.68	18.10 ± 2.54	15.02 ± 3.04	.7531
Mean largest value as increase above basal, ng/mL	.99 ± 0.10	0.70 ± 0.10	0.70 ± 0.11	.4799
Baseline characteristics :				
Valleys, #/550 min	3.89 ± .69	4.10 ± .66	4.57 ± .79	.8062
Mean width, min	60.04 ± 11.89	41.73 ± 11.28	53.76 ± 14.56	.5349
Mean concentration, ng/mL	.36 ± .05	.32 ± .04	.39 ± .06	.5864
Mean nadir concentration, ng/mL	.27 ± .03	.26 ± .03	.27 ± .04	.9582

[a] Due to high episodic frequency, episodic secretion in 6 gilts could not be clearly established by cluster analysis or visual appraisal of episodes; therefore, cluster analysis was only applied to the remaining gilts, while sliding window analysis (Table IV.3) was applied to all gilts.

[b] Least square means ± SE.

[c] Cluster analysis parameters: nadir, 2; peak, 1; upstroke *t*-statistic, 2; downstroke *t*-statistic, 2; minimum value for a pulse, .05 ng/mL

[d] Gilts in the HE group had ad libitum access to the high-energy diet until slaughter at ≈ 160 days of age. Gilts in the LE group were fed restrictively after 50 kg (≈ 80% of ad libitum) the low-energy diet until slaughtered at ≈ 160 days of age, or were then allowed ad libitum access to the low-energy diet until slaughtered at ≈ 175 days of age (LER).

[e] Means within a row lacking a common superscript letter differ.

Table IV.5. Effect of dietary regimen on characteristics of reproductive development in pre-pubertal gilts[a]

Item	HE[b]	LE[b]	LER[b]	
n	17	14	14	$P > F^c$
Reproductive tract weight, g	216.64 ± 27.02	194.27 ± 34.63	223.60 ± 34.63	.2267
Total follicular volume of the 10 largest follicles, μL	1000.72 ± 196.77	919.73 ± 206.77	1051.10 ± 206.77	.8971
Plasma estradiol-17β, pg/mL	33.112 ± 2.63	25.01 ± 2.75	29.12 ± 2.75	.1326
Follicular fluid estradiol-17β, ng/mL[d]	4.49 ± .56	3.98 ± .67	4.22 ± .67	.4592

[a] Least square means ± SEM.

[b] Gilts in the HE group had ad libitum access to the high-energy diet until slaughter at ≈ 160 days of age. Gilts in the LE group were fed restrictively after 50 kg (≈ 80% of ad libitum) the low-energy diet until slaughtered at ≈ 160 days of age, or were then allowed ad libitum access to the low-energy diet until slaughtered at ≈ 175 days of age (LER).

[c] Means within a row lacking a common superscript letter differ.

[d] Pooled follicular fluid of the 10 largest follicles.

Table IV.6. Effects of dietary regimen on characteristics of episodic GH secretion in pre-pubertal gilts as established using sliding window analysis[a].

Item	HE[b]	LE[b]	LER[b]		
n	11	11	11	SEM	$P > F$[c]
Profile mean, ng/mL	1.03	1.24	1.06	± .12	.3750
Profile maximum, ng/mL	1.35	1.64	1.28	± .16	.2764
Profile minimum, ng/mL	.76	.92	.74	± .07	.2273
Profile mean area, ng/(mL·60 min)	.96	1.15	1.05	± .05	.3479

[a] Sliding Window analysis parameters: window size, 1 h; sampling frequency, 6/h; substraction for baseline, 1.5 ng/mL

[b] Gilts in the HE group had ad libitum access to the high-energy diet until slaughter at ≈160 days of age. Gilts in the LE group were fed restrictively after 50 kg (≈80% of ad libitum) the low-energy diet until slaughtered at ≈160 days of age, or were then allowed ad libitum access to the low-energy diet until slaughtered at ≈175 days of age (LER).

[c] Means within a row lacking a common superscript letter differ.

Table IV.7. Effect of dietary regimen on characteristics of GH secretion in pre-pubertal gilts as established by cluster analysis[a]

Item	HE[b]	LE[b]	LER[b]		
n	11	11	11	SEM	$P > F$[c]
Profile mean concentration, ng/mL	1.03	1.23	1.03	± .11	.3408
Profile total area, ng/mL • 360 min	557.73	664.70	554.72	± 58.74	.3406
Episodic characteristics :					
Peaks, #/550 min	2.27	2.45	1.40	± .35	.1265
Interval between peaks, min	183.13	168.55	215.00	± 27.92	.6381
Mean width, min	46.95	48.03	45.92	± 3.56	.9171
Mean height, ng/mL	2.54	2.78	3.11	± .32	.4693
Maximum height/pre-peak nadir, %	301.45	291.78	346.21	± 32.09	.4671
Mean height/pre-peak nadir, %	172.85	176.65	195.32	± 10.57	.3123
Mean area, ng/(mL • width)	44.88	48.35	50.66	± 9.55	.9153
Mean largest value as increase above basal, ng/mL	1.60	1.74	2.20	± .30	.3696
Baseline characteristics:					
Valleys, #/550 min	1.55	1.64	1.70	± .37	.1770
Mean width, min	132.38	111.88	174.00	± 26.43	.3646
Mean concentration, ng/mL	1.00	1.11	.96	± .11	.6323
Mean nadir concentration, ng/mL	.68	.83	.71	± .10	.5811

[a] Cluster analysis parameters: nadir, 2; peak, 2; upstroke *t*-statistic, 2; downstroke *t*-statistic, 2; minimum value for a pulse, 1.5 ng/mL.

[b] Gilts in the HE group had ad libitum access to the high-energy diet until slaughter at ≈ 160 days of age. Gilts in the LE group were fed restrictively after 50 kg (≈ 80% of ad libitum) the low-energy diet until slaughtered at ≈ 160 days of age, or were then allowed ad libitum access to the low-energy diet until slaughtered at ≈ 175 days of age (LER).

[c] Means within a row lacking a common superscript letter differ.

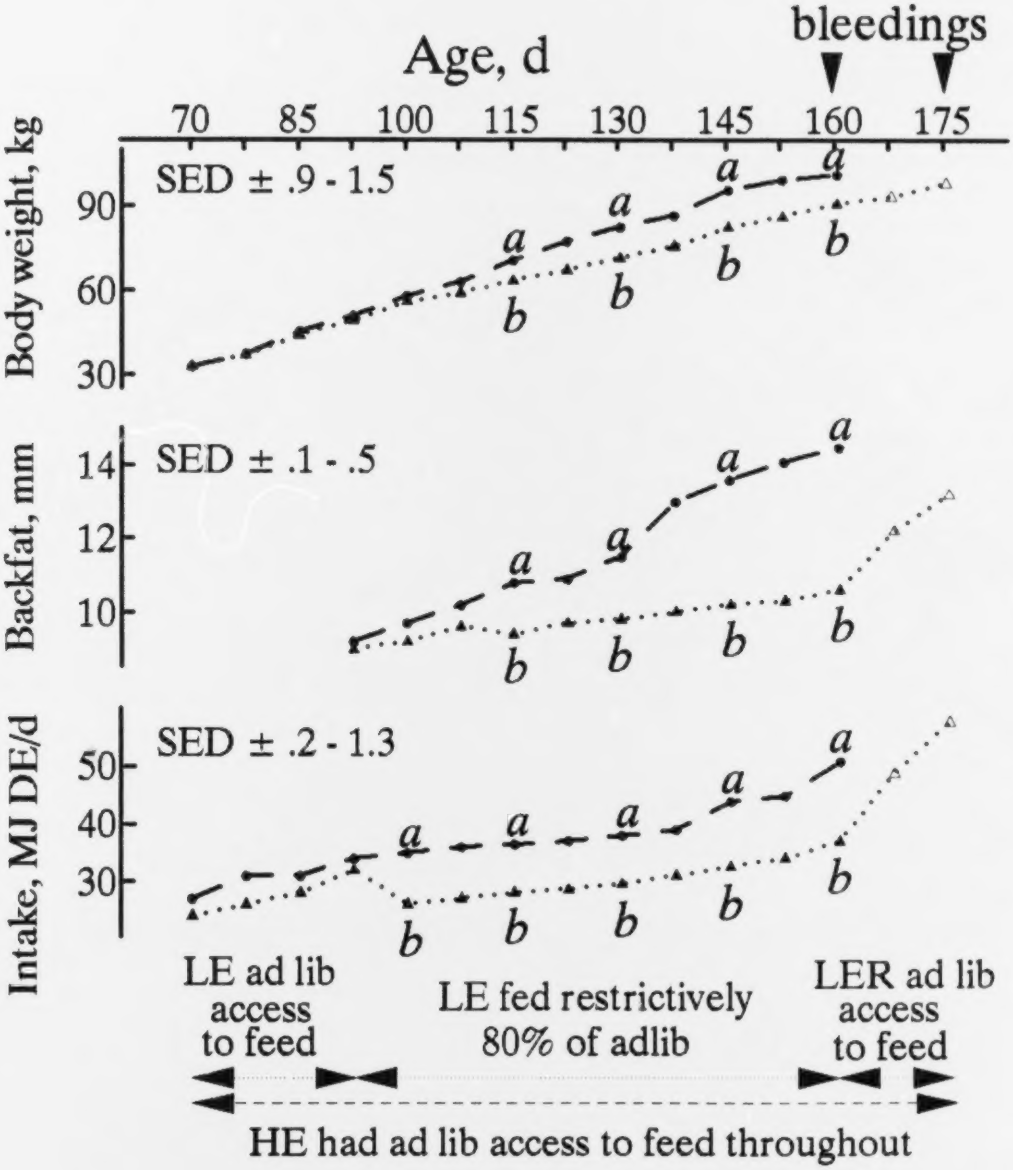

Figure IV.1. Weekly least-square means for body weight gain and backfat thickness gain and daily energy intake of pre-pubertal gilts. Gilts in the HE group had ad libitum access to the high-energy diet until slaughter at ≈ 160 days of age (dashed line). Gilts in the LE group were fed restrictively after 50 kg (≈ 80% of ad libitum) the low-energy diet until slaughtered at ≈ 160 days of age (dotted line), or were then allowed ad libitum access to the low-energy diet until slaughtered at ≈ 175 days of age (LER). Treatment profiles for a trait lacking a common superscript letter differ ($P < .05$).

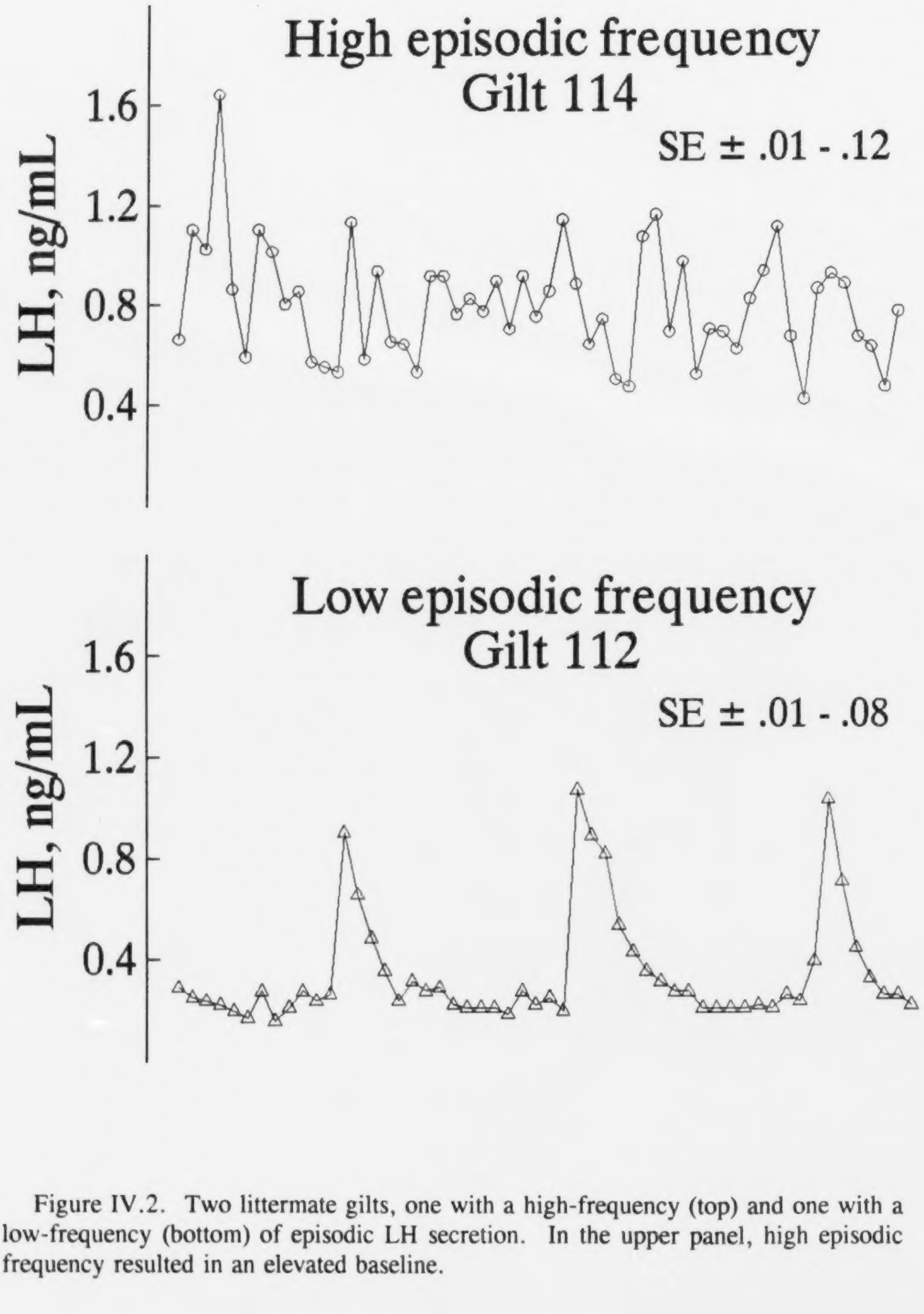

Figure IV.2. Two littermate gilts, one with a high-frequency (top) and one with a low-frequency (bottom) of episodic LH secretion. In the upper panel, high episodic frequency resulted in an elevated baseline.

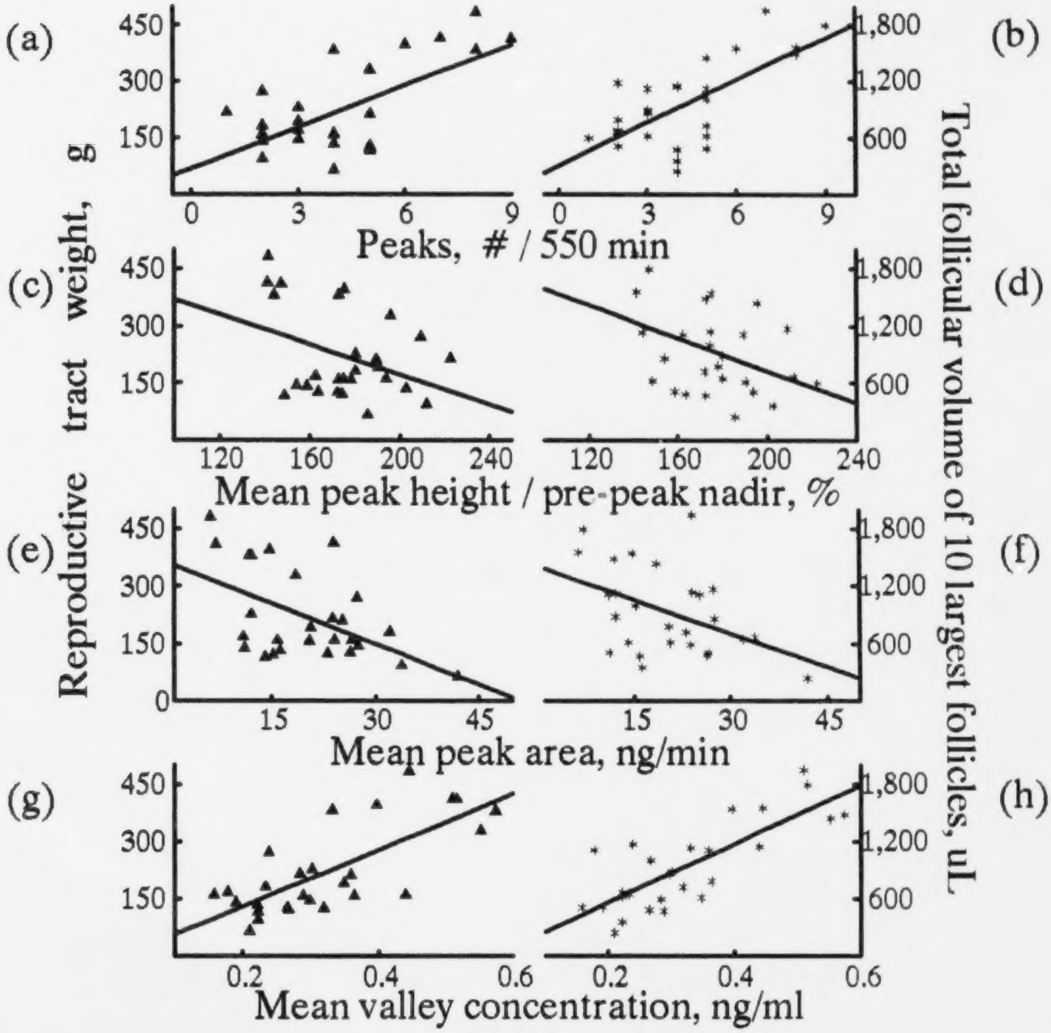

Figure IV.3. Relationships between reproductive tract weight (RTW), total follicular volume (TFV) of the 10 largest follicles and indicators of basal and episodic LH secretion: number of LH episodes (a and b), episodic LH amplitude (c, d, e and f) and basal (g and h) LH secretion. Only gilts with low episodic frequency as estimated by cluster analysis (n = 27; $P < .01$). (a), Y = 67.9032 {± 91.5863} + 36.3415 {± 8.6512} * RTW; (b), Y = 306.2068 {± 339.9621} + 151.1275 {± 32.1128} * TFV; (c), Y = 568.0100 {± 110.9894} - 1.9829 {± .9867} * RTW; (d), Y = 2445.2467 {± 425.0507} - 8.5839 {± 3.7786} * TFV; (e), Y = 361.8389 {± 102.1150} - 7.0848 {± 2.3225} * RTW; (f), Y = 1402.7402 {± 420.0255} - 23.1784 {± 9.5532} * TFV; (g), Y = -17.9964 {± 80.4389} + 736.3222 {± 133.7989} * RTW; (h), Y = -53.6479 {± 285.8113} + 3070.1834 {± 475.4071} * TFV.

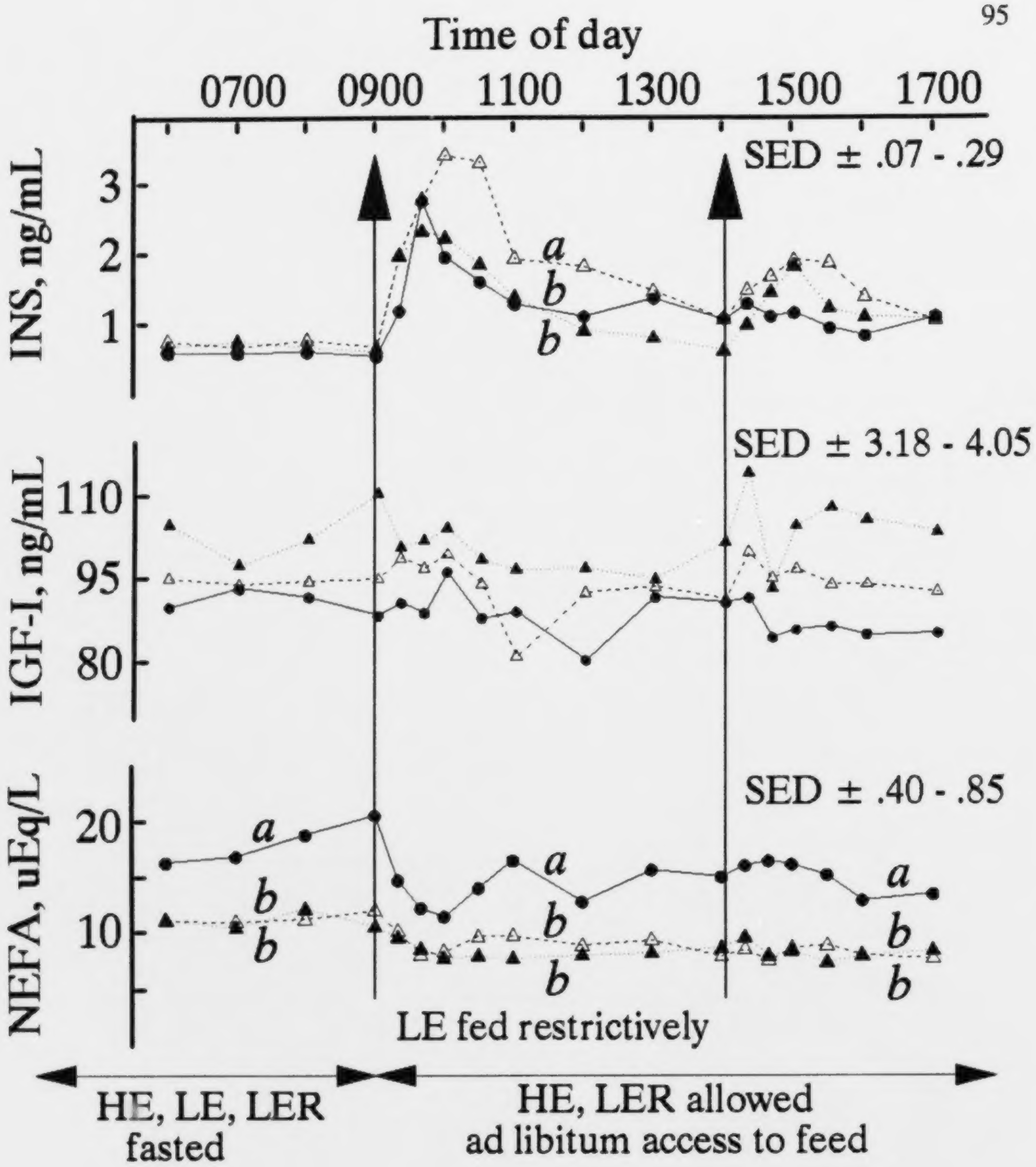

Figure IV.4. Least-square means for plasma insulin (INS), insulin-like growth factor I (IGF-I) and non-esterified free fatty acids (NEFA) concentrations for LE (dotted line), HE (solid line) gilts at 158 days of age and for LER gilts (dashed line) at 173 days of age. Except for an overnight fast preceding bleeding, gilts in the HE group had ad libitum access to the high-energy diet until slaughter at ≈ 160 days of age. Gilts in the LE group were fed restrictively after 50 kg the low-energy diet until slaughtered at 160 days of age, or were then allowed ad libitum access to the low-energy diet until slaughtered at ≈ 175 days of age (LER). Treatment profiles for a metabolite lacking a common superscript letter within a column differ ($P < .05$).

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V. METABOLIC AND REPRODUCTIVE STATUS OF PRE-PUBERTAL GILTS TREATED WITH EXOGENOUS PORCINE SOMATOTROPIN[4]

A. Introduction

In Chapter IV, a model that used nutritional strategies to manipulate the rate of fat deposition beyond the plateau in rate of protein deposition was described. This approach was used to study the effects of manipulation of body fatness on the activity of the hypothalamic-hypophyseal-ovarian axis in relation to sexual development. Nonetheless, in the relatively immature, fast-growing replacement gilt, the rate of protein accretion, rather than the rate of fat deposition, may be more important to reproductive development. It may be that fast-growing gilts achieve the genetically-determined plateau in the maximal rate of protein accretion at an older age and a heavier weight. In contrast to the rate of fat deposition, which is increasing in the growing replacement gilt, manipulation of the maximal rate of protein accretion beyond its normal expression is not possible by nutritional means. Exogenous administration of pituitary- or recombinantly-derived porcine somatotropin (pST), however, offers the opportunity to study the effects of further increases in the rate of protein accretion beyond what is achieved naturally, and therefore, the possibility to study the effects of increased lean growth on the rate of sexual maturation.

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[4] A version of this chapter has been submitted for publication. Beltranena, E., A. L. Schaefer, F. X. Aherne and G. R. Foxcroft. 1992. J. Anim. Sci.

Irrespective of its effect on body tissue repartitioning, it is important to determine what effects pST administration may have on gonadotropin secretion and gonadal development. It has been suggested that if pST administration impairs gonadotropin secretion and(or) testicular steroid production, it would suppress boar taint and boars could be raised instead of barrows, thereby resulting in more efficient pork production (see Hagen et al., 1991). In contrast to the meat-producing gilt, a similar effect in the replacement gilt would have detrimental consequences to reproductive development. Although it has been shown that growth hormone enhances the action of gonadotropins on cultured follicular tissue in vitro (Jia et al., 1986; Hsu and Hammmond, 1987), in pST-treated gilts, progesterone accumulation by cultured granulosa cells (Bryan et al., 1989) and androstenedione and estradiol-17β concentrations in follicular fluid (Hagen et al., 1990) were suppressed. These results suggest that pST administration impairs follicular steroidogenesis. Furthermore, data to demonstrate effects of pST on the attainment of puberty (Bryan et al., 1989; 1990) and ovulation rate (Kirkwood et al., 1988a, 1988b, 1989a) remain equivocal, even though embryo survival in bred gilts seemed unaffected (Andres et al., 1991; Terlouw et al., 1991). To study the mechanism(s) by which pST might affect attainment of puberty, the metabolic and reproductive status of untreated gilts and gilts given daily pST injections or prolonged-released pST implants were compared.

B. Experimental Procedure

Animals, Treatments and Diet. This endocrine study was conducted concurrently with an efficacy trial involving 60 castrated males and 60 gilts of Yorkshire breed; the methodology for the main efficacy trial is described elsewhere (Schaefer et al., 1992). At 61.7 SD 3.7 kg body weight and 128.3 SD 3.0 days of age, 23 additional gilts of the Yorkshire breed from the Agriculture Canada Lacombe Research Station herd were allocated with respect to weight and, when possible, to litter of origin to one of three treatments: Gilts served either as untreated controls (control, n = 8), or received a daily subcutaneous injection of 2 mg pST (CP 115409, Monsanto, St Louis, MO; injected, n = 8) or an ear implant devised for the prolonged-release of 2 mg/day pST (CP 115409-F, Monsanto, St. Louis, MO; implanted, n = 7). Throughout the study, gilts had ad libitum access to a pelleted diet formulated to provide a minimum of 17% crude protein, 1.1% lysine and 13.0 MJ DE/kg and fortified with vitamins and minerals (NRC, 1988).

Housing, Surgery and Bleeding. Gilts were initially grouped as duos or trios in pens with solid, heated concrete floors and wood shavings as bedding. After a 6-hour fast, one-half of the gilts in each treatment, received indwelling jugular catheters (i.d. 1.58 mm, o.d. 3.18 mm; Silastic, Dow Corning Corporation, Midland, MI) under general anaesthesia (12 mg azaperone + 75 mg metomidate hydrochloride per 20 kg body weight, i.v.; Stresnil + Hypnodil, respectively; Janssen Pharmaceutica, Mississauga, ON) on days 21 or 22 of treatment. Gilts were immediately returned to individual pens in the same area, and had ad libitum access to feed. Overnight access to feed was denied from 1900 hours on the day after surgery. Blood samples were then collected the following day at 10 minutes intervals from 0600 to 1500 hours, and ad libitum access to feed resumed immediately after the 0900-sample. Injected gilts received their pST injection after collection of the 1500 hour sample. Additional blood samples were collected from all groups at 1600, 1800, and 2200 hours (post-injection period). Plasma was harvested after centrifugation of samples (1500 x g, 15 minutes) and stored at -35°C until assayed. Surgery and bleeding was conducted over four consecutive days having divided the gilts into two group with treatments equally represented in each group; All gilts were slaughtered as one group at the Lacombe Research Station meat laboratory on day 28 of treatment. Gilts in the injected group received pST injections until the day prior to slaughter. Prolonged-release implants were recovered at slaughter from pST-implanted gilts and drug release was quantified.

Measurements. Individual body weight was recorded weekly. Feed intake was also recorded weekly, initially on a pen basis, and then after surgery on an individual basis. Backfat and loin depth measurements were taken using the ultrasonic Krautkramer USM2 (Krautkramer, Cologne, Germany) and real-time Aloka ECHO (SSD-210 DX; Osaka, Japan) probes in the live animal and on warm carcasses. Sites, at both sides of the midline were defined as: 1) loin, 5 centimetres off the midline, at a point aligned with the root of the curve of the last rib; 2) back, 5 centimetres off the midline and 5 centimetres in front of the stifle joint of the hind leg; and 3) grade, 7 centimetres off the midline, between the third and fourth last ribs.

During evisceration, reproductive tracts were collected and placed on ice. The kidneys, the inter-visceral omental leaf lard located ventrally to the kidneys, and the head were removed and weighed separately. The hot, split half carcasses were weighed and graded using the Hennessi grading system (Hennessi and Chong Ltd., New Zealand). Grading site was as defined for the live measurement grade, on the hot, left, half carcass (Anonymous, 1986).

The uterus and attached ovaries were also collected at slaughter and immediately placed on ice. After transport to the laboratory, the uterus was sectioned immediately posterior to the cervix, dissected from the broad ligament, fallopian tubes and ovaries, and was then weighed. The removed ovaries were then again placed on ice. Within the next hour, follicular fluid from the largest 10 follicles from each ovary, was aspirated and measured using Hamilton micro-syringes, pooled, snap-frozen and stored at -35°C until assayed.

Radioimmunoassays. All radioimmunoassay procedures used to assay hormone concentrations of plasma samples collected in this experiment are described in Chapter VI.B; therefore, only inter- and intra-assay coefficient of variations for each hormone are given here.

Plasma samples collected every 10 min from 0600 to 1500 hours were assayed for luteinizing hormone (LH) concentrations; samples were quantified in three assays with intra- and inter-assay coefficients of variation of 7.8 and 9.2 %, respectively.

All plasma samples were assayed for growth hormone (GH); plasma samples were quantified in two assays with intra- and inter-assay of 4.49 and 8.34%, respectively.

Plasma collected pre-prandially at 0600, 0700, 0800 and 0900 hours, post-prandially at 0920, 0940, 1000, 1030, 1100, 1200, 1300, 1400 and 1500 hours and after pST injection at 1600, 1800 and 2200 hours were assayed for insulin, and insulin-like growth factor I (IGF-I) concentrations. Plasma samples assayed for insulin were analyzed in one assay with an intra-assay coefficient of variation of 3.6%. Plasma samples assayed for IGF-I were quantified in two assays with intra- and inter-assay coefficients of variations of 3.4 and 6.9%, respectively. Pooled, follicular samples of the largest 10 follicles were also assayed for IGF-I; samples were quantified in one assay with an intra-assay coefficients of variation of 5.7%.

Non-esterified fatty acid (NEFA) concentrations were quantified in all pre- and post-prandial plasma samples and in one pooled post-injection sample; plasma samples were analyzed in six assays with intra- and inter-assay coefficients of variation of 5.3 and 13.0%, respectively.

Plasma samples collected at 0700 and 1030 hours were assayed for creatinine and urea nitrogen; samples were quantified in single assays with intra-assay coefficients of variation of 3.2 and 2.8%, for creatinine and urea nitrogen, respectively.

Individual pooled plasma prepared from equal volumes of plasma samples collected at 0700, 1100 and 1400 hours was assayed for estradiol-17β concentrations; plasma samples were analyzed in one assay with intra-assay coefficients of variation of 8.5%.

Individual pooled follicular fluid samples from the largest 10 follicles were assayed for estradiol-17β concentrations; plasma samples were analyzed in one assay with intra-assay coefficient of variation of 5.9%.

Statistical Analyses. Statistical analyses were performed using the General Linear Models procedures of SAS (1990). Differences in growth rates, feed intake, feed conversion efficiency and carcass characteristics were assessed by unbalanced analysis of variance. In order to quantify the episodic secretion of LH and GH, data were subjected to cluster (Veldhuis and Johnson, 1986) and sliding window (Shaw and Foxcroft, 1985; Foxcroft et al., 1988) analyses; visual appraisal of individual profiles was used to confirm episodic secretion (Shaw and Foxcroft, 1985; Foxcroft et al., 1988). Treatment differences for episodic characteristics of LH and GH, reproductive tract weight, follicular fluid volume, plasma estradiol-17β and follicular fluid estradiol-17β and IGF-I concentrations, were also assessed by unbalanced analysis of variance. Treatment differences for plasma concentrations of metabolic hormones, non-esterified fatty acids, creatinine and urea nitrogen over time, were assessed with time and its two-way interactions added as subplot effects, and gilt nested within treatment as the error term. Day of surgery or bleeding was initially considered as an additional effect; however, because this proved nonsignificant ($P > .05$), it was removed from the model. Comparisons between treatment means were performed by Fisher's least-significant difference test. Associations between characteristics of reproductive development and episodic LH secretion were explored using Pearson correlation analysis.

C. Results

Individual feed intake for all gilts during the 24 hours period after surgery was not different (hereafter $P > .05$) from their daily feed intake for the preceding week.

Growth Traits and Carcass Characteristics. As shown in Table V.1, there were no effects of either method of pST treatment on growth rate, feed intake or feed conversion efficiency. Similarly, pST administration had no effect on carcass weight, fat, lean, index or inter-visceral leaf lard weight. However, pST-injected, but not -implanted gilts, had a greater carcass yield than control gilts ($P > .05$).

Metabolic Status. As shown in Table V.2 and V.3, pST administration affected episodic endogenous GH secretion. Episodic mean amplitude and basal concentrations were elevated in pST-implanted gilts in comparison with control or pST-injected gilts, indicating that the implants were effective in sustaining an elevated GH baseline. Furthermore, pST administration reduced both the duration and number of GH episodes, irrespective of the method of pST administration. Despite these effects on basal concentrations and episodic frequency, pST administration did not increase episodic amplitude during the pre- and post-prandial bleeding period.

Treatment mean profiles for GH, insulin, IGF-I and NEFA based on hourly sampling during the pre-prandial, post-prandial and post-injection periods are depicted in Figure V.1. As for data from samples collected every 10 minutes, pST-implanted gilts had greater plasma GH concentrations than control or pST-injected gilts during the pre- and post-prandial periods ($P < .0694$ and $P < .0065$, respectively; Figure V.1). Following injection, however, pST-injected gilts had greater plasma GH concentrations than either control or pST-implanted gilts ($P < .0009$); concentrations of GH increased sharply, reaching a maximum one hour after injection (1500 hours) and then declined to basal concentrations by midnight.

Pre-prandial plasma insulin was not different among treatment groups ($P < .1905$; Figure V.1). Insulin concentrations increased sharply after resumption of feeding at 0900, reaching a maximum by 1000, and then declined in all treatment groups. During this post-prandial period, pST-implanted gilts showed greater and more sustained insulin concentrations than either control or pST-injected gilts ($P < .0313$). After pST injection, insulin concentrations for the pST-injected gilts increased temporarily above those of pST-implanted gilts, and for the remainder of the post-injection period, both pST-treated groups maintained greater insulin concentrations than control gilts ($P < .0170$). Plasma IGF-I concentrations were greater for both pST-treated groups during the pre-prandial and post-injection periods ($P < .0374$ and $P < .0212$, respectively; Figure V.1), yet not different from those of control gilts during the post-prandial period ($P > .3463$). For all treatment groups, plasma concentrations of NEFA were elevated during the pre-prandial period because of fasting, then declined sharply one hour after feeding and continued at basal concentrations during the remainder of the post-prandial and the post-injection periods (Figure V.1). There was no effect of pST-treatment on plasma concentrations of NEFA for the pre-, post-prandial or post-injection periods.

There was also no effect of pST administration on plasma urea nitrogen (controls, 34.85 ± 3.75; pST-injected, 33.86 ± 3.75; pST-implanted, 34.64 ± 4.01 mg/dL) or creatinine (controls, .59 ± .02; pST-injected, .58 ± .02; pST-implanted, .58 ± .01

mg/dL). There was, however, a significant effect of time ($P < .0042$) and a treatment x time interaction for urea nitrogen concentrations ($P < .0051$). Pre-prandial concentrations of urea nitrogen (0700 h; 38.26 ± 1.67 mg/dL) were greater than those post-prandially (1030 h; 30.64 ± 1.67 mg/dL). The decline in urea nitrogen from pre- to post-prandial concentrations was significantly greater for pST-injected gilts (43.39 to 24.32 ± 2.82 mg/dL) than for pST-implanted (37.35 to 31.93 ± 3.01 mg/dL) or control gilts (34.04 to 35.66 ± 2.82 mg/dL).

Reproductive Status. As shown in Table V.3 and V.4, pST administration had no effect on LH secretion. Basal LH secretion and episodic LH amplitude were similar among treatment groups; however, pST-injected gilts showed a trend towards an increased episodic LH frequency in comparison with control or pST-implanted gilts ($P < .10$). Such trends, however, were not reflected in the characteristics of reproductive tract development (Table V.5); There was a lack of correlation ($P > .05$) between reproductive tract weight, total follicular fluid volume or estradiol-17β concentrations in follicular fluid or plasma and the indicators of basal and episodic LH secretion (Table V.2). There was no effect of pST administration on reproductive tract weight, total follicular volume from the largest 5, 10, 20 and 30 follicles, or on plasma or follicular fluid estradiol-17β (Table V.5). Gilts in both pST-treated groups, however, had greater IGF-I concentrations in follicular fluid than control gilts ($P < .0178$).

D. Discussion

Efficacy of Drug Administration. The lack of an effect of pST administration on growth traits and carcass characteristics for gilts in this endocrine study was not anticipated. In contrast, for the main efficacy trial (Schaefer et al., 1992), growth rate was greater for pST-injected pigs but similar for the pST-implanted and control pigs. Average daily feed intake was lowest in pST-implanted pigs, intermediate in pST-injected and highest in the control pigs. Feed conversion efficiency was lower in pST-injected pigs than in either pST-implanted or control pigs. In terms of carcass composition, pST-injected pigs showed reduced backfat thickness, fat deposition, and increased lean yield in selective cuts (ham, loin, butt) and in overall carcass. Carcass fat deposition and lean yield were more moderate in pST-implanted than in pST-injected pigs. Although data about the efficacy of prolonged release pST implants is limited, other studies have shown that pST daily injections improved growth performance, reduced age at market weight, and improved feed conversion efficiency in swine (Etherton, et al. 1987; Bryan et al., 1989; McLaren et al., 1990; Andres, et al., 1991; Terlouw et al., 1991). Furthermore, pST daily injections increased carcass lean mass with a concomitant reduction in lipid content (Etherthon et al., 1987; Bryan et al., 1989). The most likely explanation for this difference is that the daily dose of pST used in the present study was approximately one-half of the initial dose used in gilts by the others (Bryan et al., 1989; Bryan et al., 1990; Andres et al., 1991; Terlouw et al., 1991). Furthermore, in order to minimize a confounding effect of the inherent hormonal variability associated with the late peri-pubertal period (90 - 120 kg; see

Chapter VI.D), the gilts used in this study were slaughtered at a lighter weight (86 kg), thus limiting the partitioning effects of the drug on carcass characteristics. This is suggested by the small difference in carcass yield between the pST-injected and control gilts.

Effect of pST on Growth Hormone Episodic Secretion. Despite the lack of a effects on growth traits and carcass characteristics for the gilts in this endocrine study, pST had a significant effect on metabolic status measured after 23 days of treatment. This is the first study to show that chronic pST administration as a daily injection, and as prolonged-release implants, suppresses endogenous episodic GH secretion. This suppression mechanism is presently unclear, but possibly involves a negative feedback effect directly on somatotropes, or indirectly mediated by hypothalamic and(or) hepatic GH-related receptors. This effect may primarily be mediated through an increase in circulating somatostatin concentrations, which in conjunction with circulating IGF-I and insulin concentrations, may suppress somatotropic activity or growth hormone-releasing factor (see Davis, 1988).

Hyperinsulinemia Caused by pST Administration. The pST-induced hyperinsulinemia and elevated plasma IGF-I observed in the current study, are consistent with previous reports of hyperinsulinemia and hyperglycemia as a consequence of pST administration (Evock et al., 1988; Campbell et al., 1988; Gopinath and Etherton, 1989a and 1989b; Johnson et al., 1990; Evock et al., 1991). Hyperglycemia results from increased hepatic glucose production in spite of the induced hyperinsulinemia, suggesting an impairment in hepatic sensitivity to insulin, and a parallel impairment in glucose clearance rate, which also suggests peripheral tissue resistance to insulin (Gopinath and Etherton, 1989b). Magri et al. (1990) have shown recently that this impairment of insulin action and glucose metabolism by pST in porcine adipocytes is not due to changes in receptor binding nor in insulin receptor kinase activity. Furthermore, pST administration has been shown to transpose the effects of insulin administration alone on glucose oxidation and lypogenesis in swine adipose tissue (Walton and Etherton, 1986).

Administration of pST increased circulating IGF-I concentrations in the present study. Such increases appear to be pST dose-dependent (Etherton et al., 1987; Evock et al., 1988; Johnson et al., 1990) and may mediate hyperplasia and hypertrophy at tissue level (Davis, 1988). Therefore, both insulin and IGF-I seem to mediate, perhaps synergistically, the pST-modulation of nutrient partitioning away from adipose tissue and towards muscle, resulting in decreased lipid accretion and improved feed conversion efficiency (see Walton and Etherton, 1986; Clemmons et al., 1987; Davis, 1988).

Circulating NEFA concentrations have been reported to increase in pST-treated pigs (Evock et al., 1991) suggesting pST-induced lipolysis. However, in the present study, and in the studies of Chung et al. (1985) and Johnson et al. (1991), pST administration had no effect on circulating NEFA concentrations. Walton and Etherton (1986) also failed to observe an effect of pST on lipolysis in cultured porcine adipose tissue.

Circulating urea nitrogen concentrations decreased in pST-treated pigs (Chung et al., 1985; Campbell et al., 1988; Johnson et al., 1991; Evock et al., 1991; Andres et al., 1991) consistent with observed decrease in pST-injected gilts in the present study.

Campbell et al. (1988) suggested that the increased amino acid uptake and protein accretion induced by pST may lower the pool of amino acids available for deamination and subsequent oxidation at hepatic level, thus lowering circulating urea nitrogen concentrations. The lack of a similar effect for the gilts in the implanted group may be attributed to the method of drug administration.

Effect of pST on Reproductive Development. The lack of an effect on carcass characteristics in the presence of effects on metabolic status, while unexpected, provided a unique opportunity to study the effects of pST administration on reproductive function of prepubertal gilts in the absence of effects of somatic growth. Thus, even if body fatness affects reproductive function in gilts (Kirkwood and Aherne, 1985), such confounding effects were absent in gilts in the present study.

Bryan et al. (1989) first reported that pST administration impaired the attainment of puberty in gilts; nonetheless, on the basis of results from a later study, Bryan et al. (1990) concluded that a small proportion of boar-stimulated gilts may attain puberty while receiving the drug, and that following withdrawal, onset of puberty and reproductive function may be unaffected. In contrast, two recent studies (Andres et al., 1991; Terlouw et al., 1991) and the current data, suggest that pST administration does not affect sexual maturation, the attainment of puberty or subsequent reproductive function.

If pST administration influences the attainment of puberty, it may do so by impairing gonadotropin secretion and(or) follicular development. Apart from a tendency to increase the frequency of episodic LH secretion in pST-injected gilts, no conclusive effect of pST administration on the main gonadotropic cue likely to influence attainment of puberty and ovarian development was demonstrated. Kirkwood et al. (1988b; 1990a) have reported that, following an estradiol benzoate or a PMSG challenge, pST administration for nine or six days, respectively, resulted in lower mean pre-ovulatory peak LH concentrations than in vehicle-injected pre-pubertal gilts. In a different study (Kirkwood et al., 1989b), the same group reported no effect of pST injection for three days on mean serum concentrations of LH or FSH. Lately, Andres et al. (1991) have also shown no effect of pST administration (60 d) on mean pre-pubertal serum concentrations of FSH, LH or on episodic LH release in gilts. Thus, it may be that short-term pST administration limits the gonadotropic response to exogenous hormonal challenge (Kirkwood et al. 1988b; 1990a), but long-term pST administration does not impair gonadotropin secretion.

Early reports have also suggested that pST administration influences follicular development. Kirkwood et al. (1988a) first reported that pST administration increased ovulation rate in gilts. Such action was later attributed to an ovarian IGF-I-mediated increase in follicular LH receptors, which resulted in increased hCG binding by corpora lutea of pST-treated gilts (Kirkwood et al., 1990b). Nonetheless, pST injections did not increase ovulation rate in subsequent studies (Kirkwood et al., 1988b, 1989a, 1990a). Administration of pST was also associated with a high incidence of anestrus (Kirkwood et al., 1988a; 1989a) and cystic-like follicles (Kirkwood et al., 1988b, 1990a). Bryan et al. (1989) have also suggested that the reduced incidence of puberty in their first study was associated with impaired follicular development of pST-treated gilts in that progesterone accumulation by cultured granulosa cells was reduced in the presence or absence of gonadotropins. Hagen et al. (1990) reported that pST administration alone ($\geq$ 20 d), or followed by PMSG challenge, increased IGF-I concentrations in both serum and follicular fluid, did not affect serum or follicular fluid progesterone concentrations, but lowered androstenedione concentrations. These studies thus suggest that pST administration may adversely affect follicular steroidogenesis, possibly at a point beyond progesterone synthesis. However, if pST administration were to suppress estradiol-17β production, a terminal product of the follicular steroidogenic pathway, it is difficult to explain why reproductive tract weight or follicular size were not affected in the studies of Bryan et al. (1989), Bryan et al. (1990) and Hagen et al. (1990). These parameters of reproductive tract development would normally be considered sensitive measures of estradiol-17β bio-activity. Similarly, administration of pST did not affect reproductive tract weight, follicular volume, nor plasma and follicular fluid estradiol-17β concentrations in the present study, nor in the studies of Andres et al. (1991) and Terlouw et al. (1991).

The possible effects of increased IGF-I concentrations as observed in this study and those of Kirkwood et al. (1988a) and Hagen et al. (1990) on follicular development, require further study. It may be that acute pST-administration, as in nutritional flushing (see Beltranena et al., 1991), results in a hyperinsulinemia/IGF-I-mediated increase in ovulation rate (see Kirkwood et al. 1988a) and may also result in cystic-like follicles and incidence of anestrus (Kirkwood et al., 1988a; 1988b; 1990a). Chronic pST administration, which may induce a state on insulin resistance at the ovarian level, results in apparently normal follicular development (Andres et al., 1991; Terlouw et al., 1991; the current study) but follicular steroidogenesis may be impaired (Bryan et al., 1988; Hagen et al., 1990).

E. Implications

The results of this study indicate that daily pST injection and chronic, prolonged pST administration from ear implants (2 mg/day for 28 days) suppressed endogenous episodic GH secretion and elevated insulin and IGF-I status of treated gilts. Nonetheless, episodic or basal LH secretion, reproductive tract weight, follicular volume, and plasma and follicular fluid estradiol-17β concentrations were unaffected. The physiological significance of pST-induced hyperinsulinemia and increased follicular fluid IGF-I concentrations on follicular development is unclear and requires further study. We, therefore, conclude that pST administration for 28 days during the early finishing period does not impair reproductive development of pre-pubertal gilts.

Table V.1. Effects of pST administration for 27 days on growth traits and carcass characteristics in pre-pubertal Yorkshire gilts[a]

Item	Controls[b]	pST-injected[b]	pST-implanted[b]	
n	8	8	7	$P > F$[c]
Average daily gain, kg/d	.93 ± .05	.90 ± .05	.87 ± .06	.7273
Average daily feed intake, kg/d	2.74 ± .44	2.39 ± .44	2.74 ± .57	.9237
Daily feed intake/daily weight gain, kg/kg	2.92 ± .24	2.71 ± .23	3.20 ± .27	.4267
Carcass characteristics :				
Weight, kg	67.6 ± 3.33	67.1 ± 3.33	67.7 ± 3.51	.9900
Fat, mm[d]	14.3 ± .82	11.9 ± .82	13.9 ± .87	.1192
Lean, mm[d]	36.0 ± 2.22	38.0 ± 2.22	35.9 ± 2.41	.7598
Yield, %[d]	51.3^{x} ± .33	52.3^{y} ± .33	51.4^{xy} ± .35	.0562
Index[e]	96.1 ± 3.72	97.5 ± 3.72	100.6 ± 4.01	.7094
Leaf lard, kg	.60 ± .09	.60 ± .09	.51 ± .10	.7507

[a] Least-square means ± SEM.

[b] Untreated control, 2 mg pST daily injection and 2 mg/d pST prolonged-release implanted gilts, respectively.

[c] Means within a row lacking a common superscript letter differ.

[d] Hennessi grading system.

[e] Canadian Hog Carcass Grading/Settlement System, Canadian Pork Council.

Table V.2. Effects of pST administration for 23 days on characteristics of episodic GH secretion (cluster analysis) of pre-pubertal Yorkshire gilts[a,b]

Item	Control[c]	pST-injected[c]	pST-implanted[c]	
n	8	8	7	$P > F$[d]
Profile mean concentration, ng/mL	2.17^{x} ± .29	1.74^{x} ± .29	3.24^{y} ± .31	.0067
Profile total area, ng/(mL • 360 min)	1162.75^{x} ± 158.18	939.93^{x} ± 158.18	1749.05^{y} ± 169.10	.0070
Episodic characteristics :				
Peaks, #/550 min	2.63^{x} ± .36	1.50^{y} ± .36	1.14^{y} ± .39	.0256
Interval between peaks, min	157.00 ± 30.67	175.00 ± 43.38	340.00 ± 86.76	.1897
Mean width, min	97.71^{x} ± 10.63	60.66^{y} ± 13.44	60.00^{y} ± 11.36	.0457
Mean height, ng/mL	5.57 ± 1.13	5.99 ± 1.43	5.66 ± 1.21	.9727
Maximum height/pre-peak nadir, %	501.68^{x} ± 108.95	520.40^{x} ± 137.81	194.75^{y} ± 116.47	.1236
Mean height/pre-peak nadir, %	222.26^{x} ± 20.65	198.15^{xy} ± 26.12	126.17^{y} ± 22.07	.0164
Mean area, ng/(mL • width)	184.18 ± 43.84	134.95 ± 55.46	147.61 ± 46.87	.7531
Mean largest value as increase above basal, ng/mL	4.29 ± 1.04	4.44 ± 1.31	2.54 ± 1.11	.4374
Baseline characteristics				
Valleys, #/550 min	2.75^{x} ± .40	1.25^{y} ± .40	1.14^{y} ± .43	.0190
Mean width, min	57.40^{x} ± 27.68	120.83^{xy} ± 39.15	150.83^{y} ± 31.96	.1088
Mean concentration, ng/mL	1.36^{x} ± .25	1.90^{x} ± .36	2.92^{y} ± .29	.0038
Mean nadir concentration, ng/mL	.96^{x} ± .23	1.40^{x} ± .33	2.34^{y} ± .27	.0049

[a] Least square means ± SE.

[b] Cluster analysis parameters: nadir, 2; peak, 2; upstroke *t*-statistic, 2; downstroke *t*-statistic, 2; minimum value for a pulse, 1.50 ng/mL

[c] Untreated control, 2 mg pST daily injection and 2 mg/d pST prolonged-release implanted gilts, respectively.

[d] Means within a row lacking a common superscript letter differ.

Table V.3. Effects of pST administration for 23 days on characteristics of episodic GH secretion (sliding window analysis) of pre-pubertal Yorkshire gilts[a,b]

Item	Control[c]	pST-injected[c]	pST-implanted[c]	
n	8	8	7	$P > F$[d]
Profile mean, ng/mL	$2.16^x \pm .30$	$1.76^x \pm .30$	$3.23^y \pm .32$	.0098
Profile maximum, ng/mL	2.97 ± .45	2.43 ± .45	3.64 ± .49	.2168
Profile minimum, ng/mL	$1.48^x \pm .21$	$1.27^x \pm .21$	$2.85^y \pm .22$	.0001
Profile mean area, ng/(mL·60 min)	$1.62^x \pm .29$	$1.24^x \pm .29$	$2.69^y \pm .31$	.0075

[a] Least-square means ± SEM.

[b] Sliding Window analysis parameters: window size, 1 h; sampling frequency, 6/h; substraction for baseline, 1.5 ng/mL.

[c] Untreated control, 2 mg pST daily injection and 2 mg/d pST prolonged-release implanted gilts, respectively.

[d] Means within a row lacking a common superscript letter differ.

Table V.4. Effects of pST administration for 23 days on characteristics of episodic LH secretion (cluster analysis) of pre-pubertal Yorkshire gilts[a,b]

Item	Control[c]	pST-injected[c]	pST-implanted[c]	
n	8	8	7	$P > F$[d]
Profile mean concentration, ng/mL	.43 ± .05	.44 ± .05	.33 ± .05	.2036
Profile total area, ng/(mL • 360 min)	231.52 ± 24.86	244.74 ± 24.86	176.36 ± 26.58	.1685
Episodic characteristics :				
Peaks, #/550 min	2.75 ± .46	3.88 ± .46	2.57 ± .49	.1293
Interval between peaks, min	150.00 ± 13.56	101.69 ± 14.49	120.00 ± 15.65	.0726
Mean width, min	112.19 ± 10.68	76.23 ± 10.68	94.58 ± 12.33	.0837
Mean height, ng/mL	1.43 ± .20	1.16 ± .20	.86 ± .23	.1907
Maximum height/pre-peak nadir, %	628.93 ± 98.90	577.00 ± 98.90	468.45 ± 114.21	.5731
Mean height/pre-peak nadir, %	215.67 ± 14.87	207.44 ± 14.87	176.55 ± 17.17	.2305
Mean area, ng/(mL • width)	34.09 ± 4.51	22.58 ± 4.51	18.14 ± 5.21	.0732
Mean largest value as increase above basal, ng/mL	1.20 ± .19	.93 ± .19	.67 ± .22	.2188
Baseline characteristics :				
Valleys, #/550 min	3.38 ± .47	4.12 ± .47	3.14 ± .50	.3410
Mean width, min	44.23 ± 12.96	49.25 ± 12.96	56.43 ± 13.85	.8143
Mean concentration, ng/mL	.25 ± .03	.24 ± .03	.20 ± .03	.4851
Mean nadir concentration, ng/mL	.20 ± .02	.19 ± .02	.17 ± .02	.6627

[a] Least square means ± SE.

[b] Cluster analysis parameters: nadir, 2; peak, 1; upstroke *t*-statistic, 2; downstroke *t*-statistic, 2; minimum value for a pulse, 0.05 ng/mL.

[c] Untreated control, 2 mg pST daily injection and 2 mg/d pST prolonged-release implanted gilts, respectively.

[d] Means within a row lacking a common superscript letter differ.

Table V.5. Effects of pST administration for 23 days on characteristics of episodic LH secretion (sliding window analysis) of pre-pubertal Yorkshire gilts[a,b]

Item	Control[c]	pST-injected[c]	pST-implanted[c]	
n	8	8	7	*P* > *F*[d]
Profile mean, ng/mL	.44 ± .05	.45 ± .05	.33 ± .05	.1852
Profile maximum, ng/mL	.70 ± .10	.74 ± .10	.46 ± .10	.1171
Profile minimum, ng/mL	.26 ± .02	.25 ± .02	.22 ± .02	.4404
Profile mean area, ng/(mL·60 min)	.37 ± .05	.39 ± .05	.27 ± .05	.1958

[a] Least-square means ± SEM.

[b] Sliding Window analysis parameters: window size, 1 h; sampling frequency, 6/h; substraction for baseline, .05 ng/mL.

[c] Untreated control, 2 mg pST daily injection and 2 mg/d pST prolonged-release implanted gilts, respectively.

[d] Means within a row lacking a common superscript letter differ.

Table V.6. Characteristics of reproductive development in pre-pubertal Yorkshire gilts after pST administration for 27 days[a]

Item	Control[b]	pST-injected[b]	pST-implanted[b]	
n	8	8	7	$P > F$[c]
Reproductive tract weight, g	146.75 ± 9.71	163.46 ± 9.71	150.74 ± 10.38	.4625
Total follicular volume of the 10 largest follicles, μL	397.50 ± 46.47	379.62 ± 46.47	384.86 ± 49.68	.9619
Plasma estradiol-17β, pg/mL	41.50 ± 4.17	35.52 ± 4.17	47.61 ± 4.45	.1661
Follicular fluid estradiol-17β, ng/mL[d]	3.49 ± .67	4.03 ± .67	3.72 ± .89	.5687
Follicular fluid IGF-I, ng/mL[d]	25.29^{x} ± 2.27	32.50^{y} ± 2.27	35.32^{y} ± 2.43	.0178

[a] Least square means ± SEM.

[b] Untreated control, 2 mg pST daily injection and 2 mg/d pST prolonged-release implanted gilts, respectively.

[c] Means within a row lacking a common superscript letter differ.

[d] Pooled follicular fluid of the 10 largest follicles.

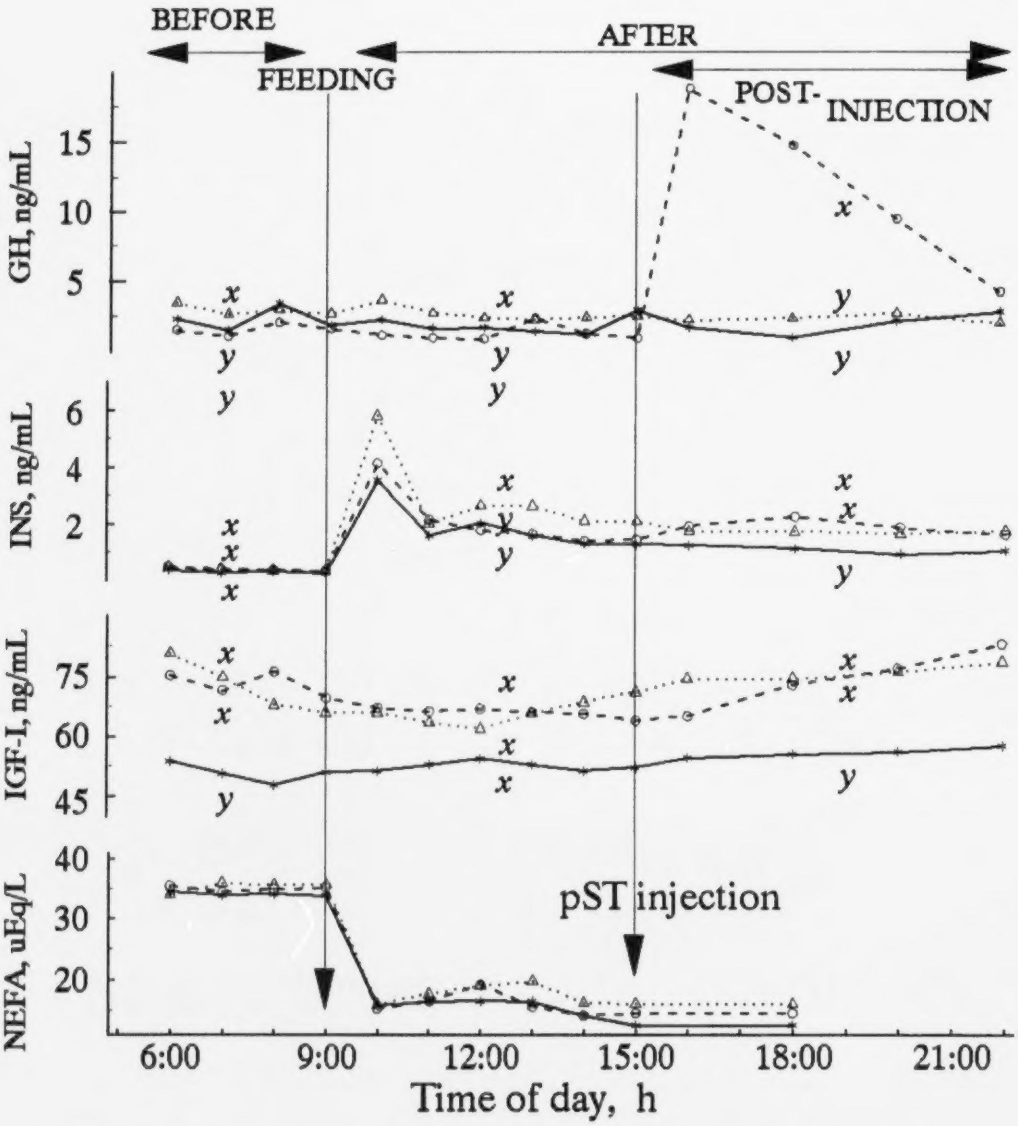

Figure V.1. Hourly mean plasma concentrations of growth hormone (GH), insulin (INS), insulin-like growth factor I (IGF-I) and non-esterified free fatty acids (NEFA) during the pre-prandial, post-prandial and post-injection periods on day 23 of treatment in untreated control and pST-treated gilts. Untreated control gilts (solid line), gilts which received a daily 2 mg pST injection (dashed line), or an ear implant devised for the prolonged-release of 2 mg/d pST (dotted line). To facilitate clarity of presentation, SED have been omitted from the profiles; these ranged as follows: GH, .43 - 1.07 (up to 14.53 ng/mL for the 1600 sample); INS, .03 - .36 ng/mL; IGF-I, 2.13 - 3.77 ng/mL; NEFA, 1.56 - 1.67 μEq/L. Treatment profile lines within a column lacking a common superscript letter differ ($P < .05$).

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VI. GENERAL DISCUSSION AND CONCLUSIONS

The relationships among body weight, body composition, growth rate and sexual development leading to attainment of puberty in the gilt were re-examined in the studies reported in this thesis.

The data described in Chapter II confirmed the suggestion of Kirkwood and Aherne (1985) and Kirkwood et al. (1987) that body weight is not a reliable indicator of sexual development, nor a good predictor of attainment of puberty. It was evident that once gilts had attained a certain state of sexual maturity that enabled them to respond to boar stimulation, body weight at puberty merely reflected body weight at the initiation of boar stimulation. If there was a permissive threshold or body weight for the attainment of puberty, this was probably below the average body weight 65 kg, at which boar stimulation was initiated in the study described in Chapter II. A similar conclusion, that body weight does not limit the attainment of puberty in modern commercial gilts with ad libitum access to feed, were made by Young et al., (1990).

The established negative curvilinear relationship between age at puberty and lifetime growth rate has practical significance for modern swine production. As discussed in Chapter II and illustrated in Figure II.2 (middle), there appears to be at least two main components in this relationship: In the first component of this relationship, with a negative slope and associated with a lifetime growth rate below .55 kg/d, the rate of somatic growth probably limits the attainment of puberty. Such low growth rates would not be commonly observed in modern swine production, but may exist in unselected lines (Bonneau et al., 1990) and in gilts fed restrictively (≈70 - 80% of ad libitum; Anderson and Melampy, 1972; den Hartog and van Kempen, 1980). In either of these situations, improvements in growth rate could be expected to result in a decrease in age at puberty. In the second component of this relationship, with a slope no different from zero and associated with a lifetime growth rate between approximately .55 and .65 kg/d, age or physiological maturity, rather than somatic growth (fat, lean), is probably limiting the attainment of puberty. Collectively, the data shown in Figure II.2 suggest that the thresholds beyond which growth rate and age will not influence the attainment of puberty in the population of gilts studied, are approximately .55 kg/d and 150 days, respectively. Any improvement in growth rate above .55 kg/d, but below .65 kg/d will probably result in gilts being heavier but not older at puberty. A third possible component was also suggested, with a positive slope and associated with a lifetime growth rate greater than .65 kg/d. In this situation, it is suggested that in the fastest, and perhaps also leanest growing gilts, attainment of puberty becomes delayed as growth rate increases; these gilts will not only be older, but also heavier at puberty, and consequently, heavier at first mating. Females represented in either the second or third component of this lifetime growth and age at puberty relationship, are likely to achieve a larger mature body size and have greater maintenance energy requirements throughout their productive life. A recommendation of feeding restrictively during the finishing period was therefore put forward for fast-growing replacement gilts: If replacement gilts would normally exceed 95 kg by 155 days of age, there is no advantage in maximizing growth rate, and therefore, gilts ought to be fed restrictively to attain this growth target. If feeding restrictively is implemented to reduce body weight at mating, then attention must be paid to the consequences of feeding restrictively on other aspects of reproductive function as discussed below.

Also of significant importance, not only to swine production but to our knowledge of growth and reproductive development, is the lack of an association between reproductive development and fatness when high levels of lean tissue accretion are achieved, as indicated from the results of the study described in Chapter IV. Caution must be exercised, however, in drawing general conclusions from these data, because at reduced or possibly very high growth rates, fatness may indeed play a significant role in regulating sexual development and the attainment of puberty. Comparing between studies, the growth rates in the population of gilts used in the study described in Chapter IV was equivalent to that in the population of gilts in the second component of age and growth rate relationship obtained in the study described in Chapter II. Thus, in gilts with reduced (<.55 kg/d) or even greater (>.65 kg/d) growth rates, puberty may be affected by body condition as follows: In the low growth rate group, the reduced growth rate may limit the acquisition of a permissive minimum level of fatness, thus being a factor preventing the attainment of puberty; this situation therefore is probably consistent with the Frisch model (see Frisch, 1988). Similarly, in gilts showing very high growth rates, as may be the case in gilts with lean genotypes, a genetically-determined delay in achieving the plateau in rates of protein deposition, and therefore the achievement of the permissive fat:lean ratio, may also result in a delay in the attainment of puberty; this situation therefore could also be consistent with the Frisch model (Frisch, 1988). It is unfortunate that in the study described in Chapter V, in which it was hoped that gilts would exceed their normal expression in protein accretion rates as a result of pST administration, the gilts did not show differences in growth or carcass characteristics, and it was not possible to describe the endocrine status associated with a measured change in body composition. For gilts which were fed to attain high lean tissue accretion levels but had their body fatness reduced or increased by dietary manipulation (Chapter IV), the observed independence of sexual development from body fatness, would appear, at first, to contradict the Frisch model and other reports in the literature (Frisch, 1988) that suggest that body fatness is an important determinant of reproductive function. Collectively, the results presented in this thesis suggest that in commercially-grown replacement gilts given ad libitum access to feed, and achieving lifetime growth rates between .55 and .65 kg/d, the extent to which level of fatness influences reproductive development is minimal. In Chapter IV, the mean level of fatness in the fat and lean groups of gilts, at the time that no difference in reproductive maturation was determined, was .12 and .14 mm backfat thickness/kg body weight, respectively (Table IV.2). Thus, the predicted threshold for an effect of fatness on the rate of sexual maturation may have been exceeded. The present results are also supported by the observed normal distribution in fatness at puberty reported by Young et al. (1990) in gilts given ad libitum access to feed. Furthermore, it must again be emphasized that most of the work on the influence of body fatness on reproductive function has been conducted in cyclic, mature, and sometimes, obese women, and female sheep and rats (see Kirkwood et al., 1987; Frisch, 1988; Foster, et al., 1988). In these situations, the level of fatness may, indeed, play a significant role in steroid metabolism and hence reproductive function. We ought to be cautious, therefore, about extrapolating between different experimental models (see Kirkwood et al., 1987; Frisch, 1988). The cyclic, mature and(or) obese rat or woman, the strenuously-physically exercising rat or woman, and the anorexic teenage girl probably represent distant metabolic states from that of the lean, rapidly growing but inactive and confined, prepubertal gilt.

From the above discussion, it is evident that greater attention ought to be given to genetic selection for precocity of sexual development. If we are to further advance the attainment of puberty in the replacement gilt, it appears that our selection efforts ought to be directed toward sexual precocity rather than to leanness and fast growth. The innate variability in gonadotropin secretion, as reported in Chapter IV, is of fundamental importance to a selection program aimed towards sexual precocity. Identification of strains, and the development of maternal lines, that show an early onset of pulsatile LH secretion, requires earnest consideration. This, in part, has probably already been achieved by the inclusion of genetic material of the precocious Chinese breeds into European lines (see Webb, 1991).

Irrespective of genetic selection programs directed toward sexual precocity, our understanding of the mechanism(s) that delays activation of the hypothalamic-hypophyseal-ovarian axis until approximately 150 days of age, when it is physiologically possible to do induce puberty at 70 days of age (see Foxcroft et al., 1989; Pressing et al., 1992), remains obscure. Since neither body weight nor fatness are limiting to the attainment of puberty in the average commercially-grown replacement gilt, other factors must be preventing activation of the hypothalamic-hypophyseal-ovarian axis at a younger age. Models of idiopathic precocious attainment of puberty and hypothalamic trauma may offer further insights to the nature of the central activation and control of gonadotropin secretion (see Terasawa et al., 1983; Stanhope and Brook, 1989).

Another objective of the work reported in this thesis was to further study the endocrine correlates of increased ovulation rate resulting from nutritional flushing. From the results described in Chapter III, it was concluded that increased plasma insulin may be an important metabolic cue, which acting in an independent or synergistic action with gonadotropins and IGF-I, mediates the ovulatory response to nutritional flushing. These results further support the evidence for short-term relationships among nutrition, metabolic state and reproduction (see Cox et al., 1987; Britt et al., 1988; Flowers et al., 1988; Booth, 1990; Downing and Scaramuzzi, 1991). The practical implication of these results is that, if growth rate in the fast-growing replacement gilt has to be restrained by feed restriction, as suggested in Chapter II, ovulation rate is likely to be suppressed. Thus, flush-feeding during the first estrous cycle, will be needed to normalize ovulation rate at mating at second estrus. Similarly a return to ad libitum feeding should be implemented for at least a two week period before exposing gilts previously fed restrictively to boar stimulation.

As mentioned before, flushing induces a short-term change in metabolic status independent of fluctuations in body composition. Our understanding of short-term changes in metabolic status has been enhanced by studying the flushing phenomenon. Of equal importance, is an understanding of the mechanisms mediating the long-term relationships between energy and(or) metabolic status and reproductive activity. These, undoubtedly encompass the mechanisms mediating some short-term relationships studied, but we have yet to understand the long-term integration of such metabolic cues and their collective, and possibly diverse, effects on the physiology of the animal's productive processes (see Porte and Woods, 1981). For example, we are yet to understand how the metabolic cues mediating tissue catabolism/anabolism in the lactating and weaned sow affect gonadotropin secretion.

Finally, and also of great relevance to modern and future swine production, are the practical implications of using tissue-repartitioning agents. In Chapter V, the effects of pST administration on sexual development were considered. No evident effect of pST administration on sexual maturation in the gilt was determined. However, further research is required to clarify the significance of pST-induced hyperglycemia/hyperinsulinemia for follicular development and steroidogenesis (see Hagen, 1990; Kirkwood and Thacker, 1991), and how pST-administration during rearing may impact on the long-term reproductive performance of the female pig.

To summarize, the results of the studies described suggest: First, that in the context of a gilt selected for and allowed to express rapid growth rate, its body weight, body fatness and growth rate have a limited influence on the attainment of puberty; attainment of the likely minimum thresholds in these traits do not therefore necessarily imply the triggering of puberty. Second, that the lack of an association between endocrine correlates of reproductive development and body fatness at high lean accretion levels, confirmed that once the threshold levels of fat deposition had been attained, body fatness does not influence reproductive development in the gilt. For the populations of gilts used in these studies the predicted thresholds, beyond which body weight, lifetime growth rate and age are not limiting the attainment of puberty are suggested as <65 kg, .55 kg/day and 150 days, respectively. Third, that increased plasma insulin may be an important metabolic cue, which in an independent or synergistic action with gonadotropins and IGF-I, mediates the ovulatory response to nutritional flushing. And fourth, that pST administration (2 mg/day) for 28 days does not impair sexual development in the pre-pubertal gilt.

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